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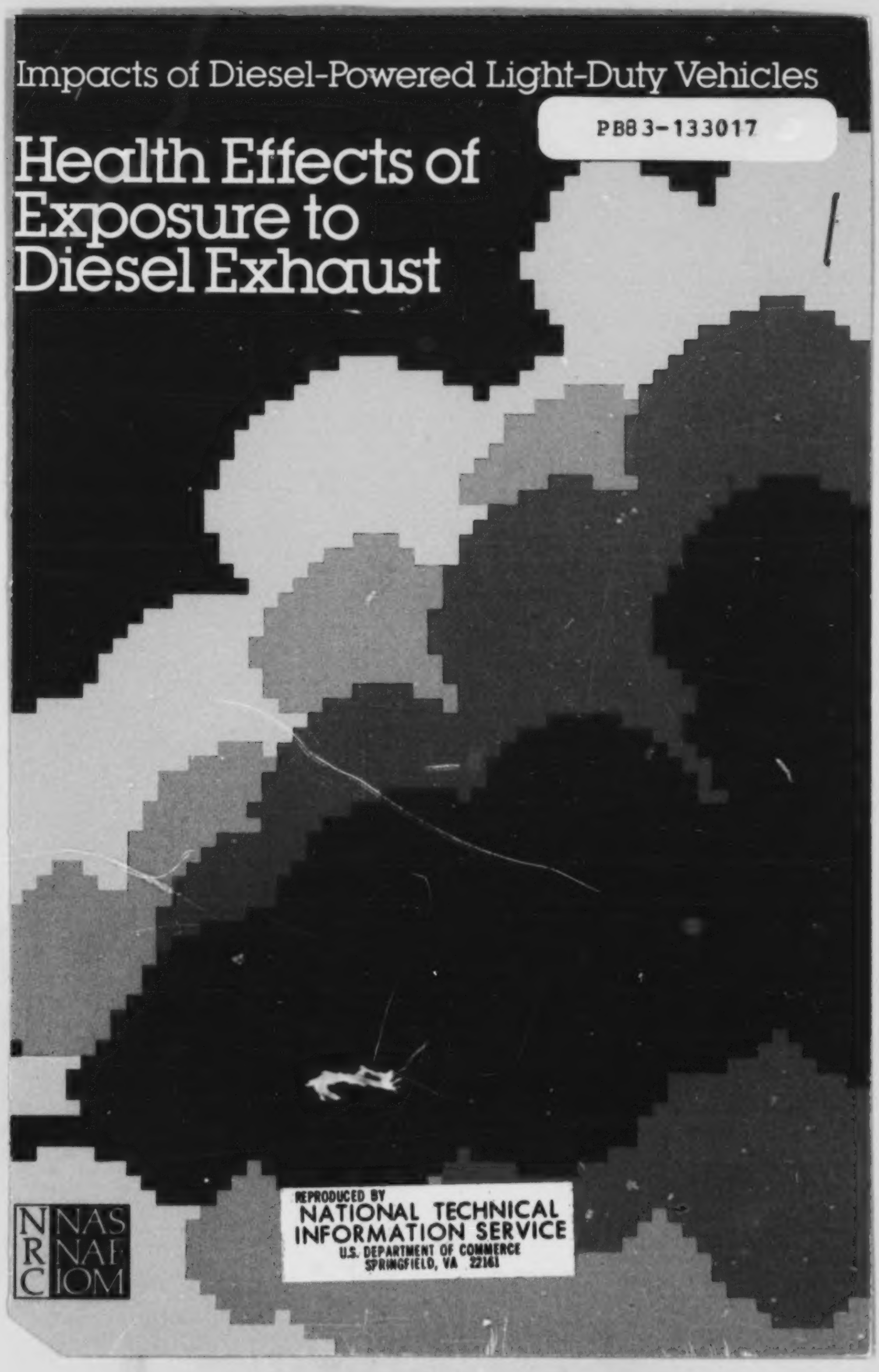

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Impacts of Diesel-Powered Light-Duty Vehicles

Health Effects of Exposure to Diesel Exhaust

The Report of the
Health Effects Panel of the
Diesel Impacts Study Committee

National Research Council

NATIONAL ACADEMY PRESS
Washington, D.C. 1981

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NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the Councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the panel responsible for the report were chosen for their special competences and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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OFFICE OF THE CHAIRMAN
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September 30, 1980

The Honorable Douglas M. Costle
Administrator of the
Environmental Protection Agency

The Honorable Charles W. Duncan
Secretary of Energy

The Honorable Neil E. Goldschmidt
Secretary of Transportation

Gentlemen:

I am pleased to transmit a report entitled Health Effects of Exposure to Diesel Exhaust, in partial fulfillment of Contract 68-01-5972 between the Environmental Protection Agency and this Academy.

The report provides a careful analysis summarizing and critically reviewing the less than satisfactory state of information concerning the effects of diesel engine emissions on humans. Materials moderately active as mutagens in various assays and as carcinogens when painted on the skins of susceptible animals have indeed been partially purified from diesel exhausts. However, no evidence of carcinogenesis has been noted in animals breathing diesel exhaust fumes or in epidemiological studies of relatively heavily exposed human populations. Unfortunately, almost all of the studies are reported to have been defective in some manner and, hence, do not permit definitive conclusions at this time. Nor do the limited observations concerning the effects of diesel exhaust emissions on pulmonary physiology, susceptibility to infection, etc., permit definitive conclusions.

However, several important, relevant studies are currently in progress. The report offers a well constructed outline of the research required to generate a degree of understanding more nearly adequate to appraise the possible impact on the public health of major transition from gasoline engines to diesel engines in the American fleet of light-duty vehicles.

Allow me to take this opportunity to express the gratitude of the Academy to Dr. Herschel E. Griffin, Chairman of the Health Effects Panel of our Diesel Impacts Study Committee, and to his colleagues for their diligence, thoroughness, and thoughtfulness in conducting this examination and preparing this report.

Sincerely yours,

P Handler

Philip Handler
Chairman, National Research Council
President, National Academy of Sciences

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PREFACE

In 1979 the Environmental Protection Agency, the Department of Energy, and the Department of Transportation requested the National Research Council to undertake an evaluation of the research and public policy issues associated with the prospective widespread use of diesel-powered light-duty vehicles in the United States. Because the Environmental Protection Agency is required under the Clean Air Act to regulate the amounts of chemical compounds and particulate concentrations emitted by all sources, including diesel vehicles, it is concerned about the possible adverse human health effects arising from an anticipated increase in the number of diesel-powered vehicles. According to projections of the nation's largest automobile manufacturer, General Motors, diesel-powered light-duty vehicles are likely to constitute 18 percent of its light-duty vehicle sales by 1985 and as much as 25 percent by the end of the century.

The Research Council's study began in May 1979 with the formation of the Diesel Impacts Study Committee, whose members were selected in accordance with its policy of providing competent experts with balanced viewpoints. Because the scope of the study involved a complex range of concerns and interactions, the committee established four panels to examine, respectively, the technological, environmental, and human health effects, and public policy issues. Each of the four panels consisted of specialists drawn from the relevant area of concern as well as some members of the committee.

Accordingly, the Health Effects Panel was formed to provide the committee with a comprehensive review and assessment of the present knowledge and understanding

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of the toxicological and epidemiological data concerning the inhalation of emissions from the tailpipes of various types of vehicles and their possible adverse health effects. Five of the panel members, including the chairman, are associated with universities, four with private enterprises, industry, or foundations, and two are engaged in government health service research. One member appointed at the start of the study, James N. Pitts, Jr., an atmospheric chemist who is Director of the Statewide Air Pollution Research Center, University of California at Riverside, resigned from the panel September 24, 1980, over his concern with the way in which the Summary and Conclusions are presented. The panel's expertise covers the disciplines of chemical analysis, mutagenesis, carcinogenesis, toxicology, biostatistics, human environmental stress, and epidemiology.

The Health Effects Panel was charged by the committee with providing an evaluation of the comprehensiveness and adequacy of health effects research on diesel exhaust emissions, and a qualitative estimate of the public health impact of uncontrolled exhaust from diesel-powered light-duty vehicles. The panel examined all of the available pertinent scientific information from academic, industrial, and governmental sources, summarizing what is known and what needs to be known. In addition, it reviewed current and planned research efforts, assessing their adequacy and comprehensiveness, as well as identifying the future research that could lead to improved and more complete data regarding the biomedical effects of exposure to diesel exhaust.

These tasks were accomplished at two panel workshops where the members heard presentations by, among others, representatives of the Department of Energy, the National Institute for Occupational Safety and Health, the Environmental Protection Agency, and the General Motors Corporation. The panel then proceeded to determine what research information was needed to ascertain the health hazards of exposure to diesel exhaust, the extent to which the information has been or soon will be provided by past and current research, and the gaps and inadequacies in those programs.

The panel judged the range of possible adverse health effects to fall within the disciplines of epidemiology, pulmonary and systemic effects, carcinogenesis, and mutagenesis. Within these categories the panel divided itself into working groups to prepare the chapters of its report.

This report considers the potential for adverse human health effects resulting from exposure to diesel exhaust. Much of the current information is derived from studies using an array of laboratory organisms. Therefore, the health effects assessment is based to a great extent on various modes of exposure and exposure routes. These include applications of whole diesel exhaust or its fractional components both *in vivo* and *in vitro*.

The inadequacy of the information base and the necessity for timely analysis of new information obtained from recently initiated research programs made the task of the panel difficult. Information and data needed to adequately assess the health effects are still being actively pursued in ongoing research. When this information is presented in the near future, the data base used by the panel will be considerably enlarged.

In the final assessment, the panel considered diesel exhaust to be the uncontrolled emission from the tailpipe of a diesel-powered light-duty vehicle. The panel recognizes that the exhaust constituents are subject to possible environmental modification, such as by photochemical oxidation, and to dispersion prior to human exposure. The possible adverse health effects from exposure to environmentally modified and dispersed exhaust are to be considered in the report of the Diesel Impacts Study Committee.

Although the Health Effects Panel has examined the information and data for determining estimates of public health risks, which are clearly factors in public policymaking, it did not establish such estimates. Rather, the Analytic Panel of the Diesel Impacts Study Committee is undertaking an assessment of the health hazards posed by the operation of a large number of diesel-powered light-duty vehicles in the future and integrating the implications of those hazards with the other risks and benefits of a large number of diesel vehicles on the nation's roads.

Thus the findings and conclusions of the Health Effects Panel are being meshed with those of the studies of the other panels--Technology, Environmental Impacts, and Analytic. All four panels will contribute to the committee's final report, which will contain the ultimate findings, conclusions, and recommendations of the entire study.

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SUMMARY AND CONCLUSIONS

In the next 20 years, if the predictions of some automobile manufacturers are right, a markedly increased number of automobiles and light-duty trucks in the United States will be powered by diesel engines. The effect of this increase in the use of diesel engines is likely to contribute to the burden of air pollution, causing problems of visibility and odor and giving rise to concern about the human health hazards from exposure to potentially harmful chemical constituents. Some of the components of diesel exhaust have been shown in laboratory tests with bacteria, animal cells, and tissues to be toxic, mutagenic, or carcinogenic. Moreover, many components are adsorbed on diesel exhaust particulates that are small enough to be inhaled and deposited deep within the lungs.

The effects of human exposure to whole diesel exhaust have not been conclusively demonstrated. Several environmental and physiological factors may bear on the potential threat of diesel exhaust to human health. Once emitted from the tailpipe, the exhaust is subject to environmental dispersion, transport, and chemical transformation--all capable of altering its components and concentrations in the ambient air at the point of human contact. Because diesel exhaust is one of many sources of air pollution, the relevant issue is the risk to human health from the incremental contribution of diesel exhaust constituents to the existing state of the air. Once inhaled, which is the primary route of intake, the health effects of diesel exhaust constituents are determined, in part, by their bioavailability. There is also the possibility of synergistic interactions between the inhaled pollutants and other inhaled substances such as cigarette smoke.

At present the environmental and physiological factors influencing the biological disposition of diesel exhaust are poorly defined. So too is the epidemiological and clinical information about the consequences of exposure. The present data base consists largely of results from laboratory experiments conducted with bacteria, mammalian cells, mammalian tissue, or live animals that were exposed through various routes to whole diesel exhaust (diluted or undiluted), to diesel exhaust particulates, to extracts of the particulates, or to gas-phase components. In many cases the conditions under which humans are exposed to diesel exhaust are generally different from those used in laboratory animal experiments. Thus, the conclusions drawn from the evidence presented by epidemiological and clinical sources may not be in complete accord with the conclusions drawn from laboratory animal data bases.

Moreover, much of the information and data received and evaluated by the Health Effects Panel are incomplete or have been drawn from unpublished papers subjected neither to the referee process nor to peer review. Therefore, the conclusions and research recommendations reached by the panel, although instructive, must be regarded as tentative and be interpreted cautiously within the context of the conditions under which the information and data were derived and presented.

The Health Effects Panel has examined the health effects of exposure to diesel exhaust in four areas--mutagenesis, carcinogenesis, pulmonary and systemic effects, and epidemiology. No epidemiological studies have been conducted on the mutagenicity of diesel exhaust. The available epidemiological information does not reveal an excess risk of human cancer of the lung or any other site in the population groups studied. This information is based entirely on occupational studies that have numerous deficiencies in research design. Only two studies approach even the minimum requirements of an adequate epidemiological study, and neither of these accounts for smoking habits. There is similarly no convincing evidence that inhaled whole diesel exhaust is mutagenic or carcinogenic in laboratory animals. However, in animal cell and whole animal skin-application tests, organic extracts of diesel exhaust particulates have been found to contain substances that have mutagenic and carcinogenic potencies similar to extracts of gasoline engine exhaust, roofing tar, and coke-oven effluent.

The apparent discrepancy between the effects of exposure to whole diesel exhaust by inhalation and the effects of diesel exhaust extracts on laboratory specimens may be due to the absence of environmental and physiological factors (dispersion, transport, transformation, bioavailability, and possible environmental synergistic, potentiative, or additive interactions) in the laboratory studies with extracts. It may also be due to differences in the amounts and routes of exposure. In the epidemiological studies, exposures to the exhaust constituents were likely to have been lower and the route of human exposure (inhalation) less direct than application of the exhaust extracts to cells, tissues, or the skin of laboratory animals. Thus, in spite of the negative evidence that has been accumulated from epidemiological studies, it is possible that diesel exhaust is carcinogenic or mutagenic in animals or humans exposed by inhalation, but at a level too low to be detected in studies conducted to date.

From available epidemiological, clinical, and laboratory animal studies, no firm conclusions can be drawn about possible pulmonary and systemic effects of diesel exhaust exposure. Although a comparatively large data base exists for pulmonary and systemic effects of certain individual gas-phase exhaust components--e.g., nitrogen oxides--there is little basis for making judgments on the cardiopulmonary effects of whole diesel exhaust--even though some of the individual components are known to exert adverse effects. However, evidence based on laboratory animal studies suggests that inhaled diesel exhaust affects the lung clearance mechanisms, produces nonspecific histopathologic changes in the lung that may or may not be reversible, and adversely affects the pulmonary defense mechanisms.

SPECIFIC CONCLUSIONS AND RECOMMENDATIONS

The specific conclusions and recommendations for future research reached in each of four areas of health effects examined in this report--mutagenesis, carcinogenesis, pulmonary and systemic effects, and epidemiology--have been arrived at with research data having recognized limitations of methodology, consistency, and certainty. The conclusions and recommendations in the four areas follow.

Mutagenesis

Evidence of genotoxic activity of diesel exhaust and hence possible carcinogenic activity is clearly demonstrated in the results obtained with short-term *in vitro* studies.

- Mutagenic compounds, both direct-acting and S9-activated, adhere to the central carbonaceous core of diesel exhaust particulates.
- Biologically active amounts of these substances may be released from particulates that are inhaled or ingested. However, based on available evidence, whole diesel exhaust does not appear to be mutagenic in mammals.
- Studies involving *in vitro* mammalian cell systems indicate that particulates in the exhaust of some diesel engines may contain sufficient levels of biologically available carcinogens to produce cell transformation under conditions of high exposure.
- In limited comparison with extracts from exhaust particulates collected from gasoline engines, diesel exhaust particulate extracts appear to contain more direct-acting bacterial mutagens. However, the activity relationships may not hold in the case of mammalian cell mutation.

Data are needed in the following areas:

- The chemical nature of the direct-acting mutagens in the extracts of diesel exhaust particulates and their potency relationships in bacterial and mammalian cells.
- The bioavailability of mutagenic and carcinogenic substances from inhaled or ingested diesel exhaust particulates.
- The transport of particulates or "biologically significant" levels of released mutagens to such critical sites as the DNA in somatic or germinal cells. Evidence for their direct interaction with DNA in covalent bonding would be most useful.
- *In vitro* studies for clastogenicity.

Carcinogenesis

In summarizing the research findings of the current experimental studies related to the potential carcinogenicity of diesel engine exhausts, it must be emphasized that much of the recent work is still incomplete. It follows that final conclusions cannot be drawn as yet. In fact, some of the most important *in vivo* carcinogenesis studies are currently in progress. Nevertheless, based on the available data, some definitive and some tentative conclusions can be drawn.

- Extracts from diesel (and from gasoline engine) exhaust particulates contain carcinogenic materials. This conclusion is supported by many older as well as current chemical and biological studies. The carcinogenic activities of these extracts appear to be two or three orders of magnitude less on a weight-to-weight basis than that of benzo[*a*]pyrene, a representative carcinogenic polycyclic aromatic hydrocarbon.
- Whether whole engine exhaust particulates (from gasoline and diesel engines) are carcinogenic is as yet unknown. Existing data are limited and are either negative or ambiguous. Important studies are under way involving lung tumor development in Strain A mice and Syrian golden hamsters.
- Whole diesel or gasoline engine exhausts have not been found so far to be carcinogenic when inhaled by laboratory animals. This negative finding is based mostly on previous studies with a variety of animal species (mice, rats, hamsters, and dogs). Chronic large-scale inhalation studies that are presently under way have not, as yet, yielded information concerning carcinogenicity.
- Variable fuel composition and engine operating characteristics may turn out to be significant determining factors in the biological activity of diesel engine exhaust materials, according to *in vitro* cell transformation assays. At the moment it is uncertain whether the *in vivo* carcinogenesis assays will show the same trends.
- Based on the skin carcinogenesis studies of Brune and coworkers (1978), Misfeld and Timm (1978), and Misfeld (1979), in which the carcinogenic activity of diesel engine and gasoline engine exhaust extracts (the engine used was not equipped with a catalytic converter) were compared, it appears that per mile traveled (or on a weight-to-weight basis) the amount of carcinogenic material emitted might be within the same order of magnitude for both engine types.

- Based on the data presently available from skin tumor-initiation studies (Slaga *et al.*, 1979), the activities of extracts of roofing tar, coke-oven effluent, and the exhaust components from one gasoline engine and two diesel engines are all within the same order of magnitude (per unit weight of material tested). It should be remembered, however, that this comparison does not take into consideration the environmental concentrations of the various effluents to which man is actually exposed.
- Mouse skin carcinogenicity data and other quantitative bioassay data can be used to estimate the relative carcinogenicity of organic extracts of both diesel exhaust and related environmental emissions. The estimates can then be combined with available epidemiological data on the related environmental emissions in attempting to assess the potential human cancer risk from exposure to diesel engine emissions. Harris (1981) has performed such an assessment. It is based upon epidemiological data on occupational exposure to coke-oven and roofing-tar emissions, along with the results of initiation-promotion experiments in mouse skin and oncogenic transformation experiments from ongoing EPA studies. The resulting estimates of the potential range of lung cancer risk are of the same order of magnitude as those obtained from an epidemiological study of lung cancer among diesel bus garage workers (See page 126 *et seq.*)

  It needs to be recognized that this method of comparative risk assessment assumes that the relative potencies of environmental emissions are preserved across human and nonhuman biological systems. This assumption is based on many unknowns. The practical value of risk assessments relying on such assumptions is limited, in view of interspecies and interorgan differences in factors such as bioavailability, particulate distribution, extractability and clearance of active organics, target site of action, metabolism, and genetic repair mechanisms.

Both past and current studies either leave a number of important questions unanswered or provide only insufficient information. Thus:

- Essentially no information is available concerning the carcinogenicity of gas-phase components. Attempts should be made to learn more about these substances.

- Further identification is needed of the components of diesel exhaust fractions that contain mutagenic and carcinogenic activity. The chemical characterization of these materials would be useful to guide future attempts at engine modification to reduce carcinogenic emissions.
- One of the major questions that has not been adequately resolved concerns the *in vivo* bioavailability of the toxic substances adsorbed to diesel exhaust particulates. In general, available information seems to indicate that the organic substances are tightly bound to the carbonaceous core. Although they are extractable with polar solvents such as methylene chloride, the evidence from several experiments suggests that these materials are not bioavailable. However, several *in vitro* studies, including one with *xeroderma pigmentosum* cells, as well as two inhalation studies, suggest that some of the materials associated with diesel exhaust particulates are bioavailable. Studies should be designed to measure, for example, the elution of polycyclic aromatic hydrocarbons from the diesel exhaust particulates *in vivo*.
- Future investigations need to place more emphasis on comparative studies of the relative carcinogenicity of light-duty diesel engine exhaust and the exhausts of gasoline engines (with and without catalytic converters). This is essential because the decisive question is whether diesel engines add more potentially carcinogenic agents to the environment per mile driven than gasoline engines under the same load.
- At present, the most quantitative carcinogenesis data may be derived from studies of skin carcinogenesis and intraperitoneal injection with Strain A mice. Future studies should also make use of another highly sensitive bioassay model--i.e., the newborn mouse (Asahina *et al*., 1972).

To make specific recommendations based on mostly incomplete data seems unwarranted. Still, three facts emerge from the various chemical and biological studies in spite of all the shortcomings of the assay systems used. They are: diesel exhaust contains traces of carcinogenic materials; the carcinogenic activity of these materials in diesel exhaust appears to be low; and variations in engine operating characteristics and fuel type appear to greatly affect the carcinogenic activity of diesel exhaust particulates.

Pulmonary and Systemic Effects

With the available information, few conclusions can be drawn regarding pulmonary and systemic effects. This is due to the paucity of information on human health and the preliminary state of the experimental work in progress.

- The acute and chronic inhalation of diesel exhaust produces, as expected, the accumulation in the deep lung of carbonaceous particulates as well as potentially hazardous compounds adsorbed to them. Such materials become sequestered primarily in alveolar macrophages and, to a limited extent, in cells of the alveolar epithelium. Clearance may occur via the mucociliary escalator and the pulmonary lymphatic system. The possible long-term consequences of such accumulation, with regard to its potential for causing chronic pulmonary disease, is a key issue in the evaluation of diesel exhaust inhalation hazards. Furthermore, there is the question of whether adverse health effects may be exacerbated if synergistic interactions occurring in the environment (e.g., those between diesel exhaust particulates and products of photochemical reactions) increase the toxicity of exhaust components. Experimental data are insufficient to resolve this question.
- Histopathological changes induced by inhaled diesel exhaust are nonspecific. They may be interpreted to reflect initial cell damage followed by recovery with discrete areas of fibrosis and possibly emphysematous changes. The current data confirm that the fibrogenic potential of diesel exhaust is low. Additional lifespan exposure data are needed.
- A single observation suggests that inhaled material may induce biochemical changes in organs distant from the respiratory tract. Because such materials are cleared relatively slowly, studies following inhalation exposure need to be of sufficient duration to determine the secondary effects of inhaled materials.
- Present information suggests that pulmonary defense mechanisms may be adversely affected by diesel exhaust. It is not clear whether the agents responsible for this phenomenon are associated with the gaseous or the particulate phase of the exhaust. Low levels of NO_x exposure have been shown to decrease resistance to infectious diseases in both animals and humans.

- Available information suggests that a single high-level exposure to diesel exhaust can produce acute toxic effects (e.g., poisoning due to NO_x, to aldehydes, and possibly to CO), whereas long-term exposure to comparatively low diesel exhaust levels has not clearly been shown to cause pulmonary and systemic toxicity. Determination of ultimate health effects requires consideration of the data bases on studies involving both acute and chronic exposures.
- Analysis of the available experimental evidence for pulmonary and systemic health effects caused by exposure to diesel exhaust suggests that it is possible to estimate the health hazards from the expected increase in gaseous and particulate components in the general atmosphere. With respect to pulmonary and systemic effects, it is reasonable to expect that the health hazards associated with certain pollutants originating in diesel exhaust (SO_x, NO_x, CO, and possibly particulate material) would be qualitatively similar to those associated with the same pollutants from such other sources as fossil-fueled power plants.

The data base is extremely limited for the pulmonary and systemic health effects of exposure to diesel exhaust. The following are the most obvious research gaps:

- Information is lacking on the acute toxicity of diesel exhaust. A reevaluation of the acute effects of diesel exhaust on lungs should emphasize exposure to exhausts with different characteristics. These are generated by varying the modes of engine operation and by using different fuels. Primary lung damage and recovery should be fully documented with quantitative morphological techniques and selected physiological and biochemical studies (airway resistance, induction of protective enzymes, etc.). None of the presently conceived studies has considered the usefulness and value of detailed cell kinetic studies. These are of particular assistance in quantitating initial cell death in the lung (Evans *et al.*, 1978).
- It is necessary to determine the possible long-term consequences of diesel exhaust inhalation, such as the development of fibrotic and emphysematous changes in the lung. One important aspect is to determine whether lesions are reversible upon cessation of exposure. In order to provide this information, different animal species should be exposed to graded concentrations of

diesel exhaust in studies of several months duration, while fully documenting the extent and degree of the induced changes.

- Additional quantitative data need to be obtained on initial deposition and clearance of inhaled particulates, possible translocation to other organs and tissues, and retention in the body. The leaching of potentially toxic compounds from the particulates must be determined and related to potential systemic effects. A single study reporting that inhaled diesel exhaust causes biochemical changes in extrapulmonary tissue (Lee *et al*., 1980) suggests the need for the additional studies.
- Increased susceptibility of animals to infection following inhalation of diesel exhaust needs to be evaluated in young, mature, and old animals. A further question is whether resistance to bacterial and viral challenge is modified primarily by the gas phase or by the particulate fraction of diesel exhaust. The functional biology of particulate-laden macrophages and the overall capacity of the macrophage system to handle and remove particulate material under conditions of continuous exposure must be thoroughly investigated. Such work would pursue cell kinetic studies on the biology of macrophages (Adamson and Bowden, 1980) and quantitative morphometric studies. The effects of diesel exhaust on the immune system in the lung and in other organs must be evaluated with appropriate techniques (Vos, 1977).
- It is important to determine how diesel exhaust affects humans with preexisting diseases. For example, the presence of pulmonary emphysema has been shown in one study to alter the deposition and long-term clearance of inhaled particulates in hamsters (Hahn and Hobbs, 1979). That no experiments are planned with animals suffering from conditions similar to certain human diseases is clearly a research gap. Such animal models exist for pulmonary emphysema, pulmonary fibrosis, immunosuppression from cigarette smoking, alveolar lipoproteinosis, chronic pulmonary hypertension, increased sensitivity to ozone, and cardiomyopathy with general heart failure. There is an urgent need to develop and use animal models of human diseases in order to relate the effects of specific primary and secondary products from diesel exhaust to the specific disorder(s) present in humans.

Epidemiology

The following conclusions can be appropriately drawn from the review of the literature on diesel exhaust exposures:

- In epidemiological studies of occupational exposure to diesel engine emissions, excess risk of cancer of the lung, or of any other site, has not been convincingly demonstrated. The evidence to date does not indicate that exposure to diesel exhaust is a serious cancer hazard, at least at exposure levels no greater than those that existed in London bus garages. Only two studies, one on railroad workers (Kaplan, 1959) and the other on bus garage workers (Raffle, 1957; Waller, 1979), approximate even the minimum requirements for a sound epidemiological evaluation of cancer risk. Both of these studies, however, suffer from numerous deficiencies in design. Hence, their negative conclusions must be viewed with caution.
- The evidence of a relation between occupational exposure to diesel exhaust and the prevalence of chronic obstructive lung disease is inconsistent. Some studies have suggested that workers exposed to diesel exhaust have a higher prevalance of chronic respiratory symptoms, bronchitis, and diminished lung function than otherwise comparable persons who have not been exposed. Other studies have failed to confirm the observations.

Future research programs should include consideration of the following items:

- Additional carefully controlled studies of populations occupationally exposed to diesel engine exhaust are needed. In such studies, both the whole exhaust and its individual components should be carefully monitored. The studies must carefully control for cigarette smoking, which plays a dominant role in the etiologies of lung cancer and chronic obstructive lung disease.
- Several epidemiological studies have suggested synergism between cigarette smoking and occupational exposure in the development of lung cancer (International Union Against Cancer, 1976). Asbestos workers and uranium miners who smoke appear exceptionally prone to develop this cancer (Archer _et_ _al_., 1973; Selikoff _et_ _al_., 1968, 1980). Synergism between cigarette smoke and diesel exhaust might similarly increase the risk of lung

cancer, although this has not yet been shown to occur. Researchers need to keep this possibility in mind in future epidemiological studies.

1 INTRODUCTION

Approximately 0.5 percent of the light-duty trucks and automobiles in operation in the United States during 1979 were diesel-powered. The automobile industry predicts that this percentage will increase markedly during the next 20 years, suggesting widespread use of diesel-powered vehicles by the year 2000. Exhaust from these vehicles may contribute to an increasing environmental burden of air pollution. This, in turn, has given rise to concern about human health hazards and the current technological capabilities to control potentially harmful constituents of diesel exhaust.

Diesel exhaust consists of many recognized toxic agents in two phases. The gas phase contains primary products such as carbon monoxide (CO), sulfur oxides (SO_x), nitrogen oxides (NO_x), and aldehydes (e.g., formaldehyde, acrolein). These influence the production in the atmosphere of secondary chemical products, such as the oxidants ozone (O_3), nitrogen dioxide (NO_2), and peroxyacetyl nitrate (PAN). The particulate phase contains small respirable carbonaceous particulates that can aggregate. These provide a surface on which a number of organic chemicals are adsorbed, most notably polycyclic aromatic hydrocarbons (e.g., benzo[a]pyrene).

There are indications that the uncontrolled exhaust from light-duty diesel engines contains a larger amount of NO_2 than does the exhaust from spark-ignition engines, and that the NO_2:NO_x ratio is higher. Moreover, SO_2 emissions may be as much as 10 times higher. Of more critical concern is that studies by the Environmental Protection Agency (EPA) have found that diesel-powered cars emit 30 to 100 times more particulates than gasoline-powered cars equipped with catalytic converters, which typically emit no more than 0.008 gram per mile.

The EPA has established new standards for particulates in exhaust emissions of 0.6 gram per mile for 1982 to 1984 models and 0.2 gram per mile for passenger car models manufactured in 1985 or thereafter.

Automobile manufacturers and research organizations in the United States and abroad face three problems: limited ability to control particulates and NO_x emissions in diesel exhaust with present technology and at reasonable cost; the variations in the properties of diesel fuels and fuel additives that lead to a broad range of combustion products, which may be altered by a combination of physical, chemical, and biological processes; and the operating characteristics of different engine types, which also influence the composition of diesel exhaust.

As with gasoline engine exhaust, several hundred different chemical compounds are likely to appear in the exhaust from diesel engines (Schuetzle et al., 1980). Some of these chemicals have been identified by animal exposure tests to be toxic, mutagenic, or carcinogenic. Many of these compounds are adsorbed on the surface of the diesel exhaust particulates, which are small enough to be inhaled and deposited deep within the lungs. Exposure of laboratory animals to diesel exhaust produces morphological and biochemical changes in the lung, increases susceptibility to bacterial infection, and may even produce systemic toxic effects. Moreover, eye irritation is caused by direct contact with diesel emissions. Thus, the central issue for the future of the diesel engine in light-duty vehicles is the possibility of its exhaust causing risk to human health.

Although several federal agencies are now conducting research on the potential health hazards of diesel exhaust particulates and of certain chemical compounds in the exhaust, the existence of adverse health effects in humans, including carcinogenesis, has not been conclusively demonstrated. Such scientific uncertainty about health effects poses a dilemma for policy makers who need to assess the health risks of exposure to diesel engine exhaust before making decisions and setting standards.

The health hazards of diesel exhaust have not been compared comprehensively with those of the gasoline engines they would replace. Secondary exhaust products arising from environmental transformations may be expected to differ quantitatively due to their induction by the higher levels of SO_2 and NO_2 emitted in diesel exhaust than in gasoline exhaust, as well as by the higher levels of ozone (O_3) in the ambient air. An additional complication is the diversity and distribution of diesel engines that contribute to ambient pollution levels--including those in stationary sources, in locomotives, in trucks, in tractors, in buses, and in automobiles.

Beyond the possible hazards to human health, diesel exhaust causes several aesthetic and environmental problems. Diesel engines produce considerably more odor, smoke, and noise than do well-maintained gasoline engines.

This report explores the information available on the possible adverse health effects caused by exposure to whole diesel exhaust or its components and examines the likelihood of current and planned research programs to provide data needed for more reliable health risk estimates. Such estimates will be determined by the Analytic Panel of the Diesel Impacts Study Committee. That panel's findings with regard to human health hazards are an extension of the information and data elucidated in this report by the Health Effects Panel.

Because of the relatively recent awareness of the issues arising from the increased use of the diesel engine, and the equally recent attempts to resolve them, much, although not all, of the information and data reviewed and evaluated by the Health Effects Panel has been derived from unpublished papers that were subjected neither to the referee process nor to peer review. The findings and results cited from many of the studies referenced in this report must therefore be viewed with reservation.

2 MUTAGENESIS

The observation, in 1978, that diesel exhaust particulates suspended in an organic solvent produced mutations in a bacterial assay was significant. It has shown not only that the extractable materials could damage deoxyribonucleic acid (DNA) but also that they might be carcinogenic (Huisingh _et al._, 1978). The latter conclusion is based on the observation that most chemical carcinogens have been shown to be mutagenic in a diverse group of submammalian and _in vitro_ mammalian assays (Ames, 1979; Ames _et al._, 1973; Bouck and di Mayorca, 1976; Brusick, 1978; McCann _et al._, 1975; Magee, 1977; Miller and Miller, 1971).

GENOTOXIC CHEMICALS AS CARCINOGENS

Chemical mutagens are toxic substances that induce alterations in chromosomal DNA. If the altered genes are located in mammalian gametes (sperm or egg cells), hereditary diseases or morphological changes might result (Brusick, 1980). In addition, it is generally assumed that genetic alterations are a critical step in the biological processes leading to cancer and teratogenic changes (Ames, 1979; Brusick, 1977; Kalter, 1971). Such effects must also be considered in mutagenesis evaluation.

Short-term submammalian and _in vitro_ mammalian tests are essentially predictive in nature and thus are not definitive tests either for carcinogenesis or teratogenesis. However, their carcinogenesis predictive coefficient is near 90 percent or greater when a battery of tests is used (Brusick, 1978; Hollstein _et al._, 1979). Therefore, such results contribute to an overall assessment of the potential for carcinogenicity. The predictive value of these tests for teratogenesis is not nearly as good. Consequently, they are not generally used in making assessments of the potential for teratogenicity (Brusick, 1977).

GENOTOXIC CHEMICALS AS MUTAGENS

The existence of a genetic hazard can be confirmed by extending the short-term test data base to include results from _in vivo_ studies. Although animal models can be used to detect genetic damage of several types, they are severely limited with respect to their application to quantitative risk assessment. _In vivo_ genetic studies, like those for carcinogenicity, must be conducted on small populations of animals and at dose levels many times higher than those found or anticipated in the environment. Animal model systems must be viewed as the best source of risk analysis data, because it is extremely difficult to establish a cause-effect relationship for a single human mutagen. Because DNA is essentially identical in structure and function in all organisms, the demonstration of genetic alterations induced by a chemical in one organism is strongly suggestive that similar damage will be induced in other organisms if it can be established that the chemical reaches the target molecule, DNA.

GENERAL CONSIDERATIONS CONCERNING MUTATION

Geneticists assume that some hereditary change in humans is a consequence of exposure to chemical or physical mutagens. Changes may be induced in transmissible gametes* or nontransmissible somatic cells (Brusick, 1980). Cause-effect relationships are difficult, if not impossible, to establish for mutational events for the following reasons:

- A mutation induced in either a male or female gamete may be recessive, and thus may not be expressed in the first generation (F_1 offspring) following its induction. This mutation may never be expressed, or it may be expressed several generations subsequent to its induction. Therefore, no cause-effect relationships can be established. Despite this limitation, the consequences of gene mutations to human health warrant attempts at pursuing the identification of potential human mutagens. Methods presently under consideration for developing the data to establish cause-and-effect relationships include monitoring humans for new dominant mutations and evaluating spontaneous or medical abortions for evidence of genetic damage. The latter approach has yielded the best evidence available showing a relation between chemical exposure and frequency of abnormal chromosomes in aborted embryos (Department of Health and Social Security of England, 1979).

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* Only those mutated cells that are able to pass through meiosis and to form zygotes leading to a live birth are considered.

- The frequency of new mutations that can be identified in the zygote, embryo, fetus, or newborn is so low that extremely large human populations must be sampled to show meaningful cause-and-effect relationships, especially under exposure conditions that only double or triple the normal background rate.
- The human genome (complete set of chromosomes) consists of approximately 50,000 genes. Mutations may affect any of these genes and consequently may produce a wide range of consequences. For this reason, it is difficult to know how to look for mutation in humans (i.e., the detection of new phenotypes). The exact frequencies of most mutations in the human population are not now known. Therefore, to look for changes in the frequency of these mutations, even to the extent of 100 percent difference, is not feasible.

EVALUATION OF DIESEL EXHAUST PARTICULATES

Short-term submammalian and _in vitro_ bioassays will be valuable tools to develop supporting data for _in vivo_ carcinogenesis studies. Their predictive nature and operational flexibility make them ideally suited to answer questions not amenable to study via conventional _in vivo_ bioassays. For example, studies comparing the biological activity of diesel exhaust particulate extracts prepared with a variety of organic solvents and biological fluids would be an impossible task using _in vivo_ bioassays. They can be readily performed, however, with a wide range of short-term _in vitro_ bioassays.

Different classes of chemicals produce different types of mutation and chromosomal damage in DNA. Therefore, several kinds of short-term tests must be used in a comprehensive evaluation of diesel exhaust particulates and their extracts. These should include gene mutations, chromosomal aberrations, and DNA repair endpoints.

In vivo studies address the issues of somatic cell and heritable genetic damage. Most important should be the analysis for induction of heritable changes in the gametes arising from inhalation exposure. The *in vivo* tests should also cover the major types of genetic endpoints mentioned above.

CHEMICAL ANALYSIS AND COMPOSITION OF DIESEL EXHAUST PARTICULATES

One characteristic that may be used to evaluate the mutagenic potential of diesel exhaust emissions is the chemical nature of the substances present. Considerable effort has been expended by a number of workers in an attempt to characterize the chemical composition of these emissions (Choudhury and Doudney, 1979; Hare and Baines, 1979; Huisingh *et al.*, 1978; Lyons, 1962; Risby and Sigsby, 1980; Rodriguez *et al.*, 1979; Williams and Chock, 1979; U.S. EPA, 1980; Zweidinger *et al.*, 1980a, 1980b). Attention has been focused on the particulate fraction generated, where mutagenic activity appears to reside. Some mutagenic activity also has been detected in the gaseous fraction (Bradow, 1980; Lofroth, 1979). The results of these studies reveal a novel spectrum of mutagenic activity, and thus preclude the possibility of establishing simple correlations relating the mutagenic potential of diesel exhaust to that of other combustion products such as cigarette-smoke condensate and coke-oven emissions (Claxton, 1979; Claxton and Barnes, 1980; Nesnow and Huisingh, 1979; Wei *et al.*, 1980).

During the past 20 years, a broad range of studies has established that most chemical mutagens and carcinogens exert their effects through the chemical modification of DNA (Brooks, 1977; Grover, 1976; Lutz, 1979; McCann *et al.*, 1975). Alkylating agents that are direct-acting and those that are generated from parent substances through the action of enzymes located in the endoplasmic reticulum of hepatocytes and other cells form covalent bonds with DNA. These latter substances have been termed "indirect-acting" mutagens or promutagens because their mutagenic activity is dependent on metabolic conversion to the chemically reactive alkylating species catalyzed by enzymes present in the S9 mix[*] (National Research Council, 1972, 1976). A classic example of a promutagen is the polycyclic aromatic hydrocarbon, benzo[*a*]pyrene. As discussed below, the mutagenicity of diesel exhaust differs from that of these indirect-acting agents because metabolic activation of the type required for benzo[*a*]pyrene cannot be demonstrated.

Early studies on the particulate fraction from diesel engine exhaust indicated the presence of substances that are mutagenic in the Ames mutagenesis assay. Recent work has characterized the particulates as nonmutagenic (Risby and Sigsby, 1980), although they were shown to carry mutagenic materials that can be eluted by such hydrocarbon solvents as methylene chloride and benzene-methanol. The complexity of the composition of these extracts has been partially documented by gas chromatographic procedures and mass spectrometry. The analyses indicate that perhaps as many as several hundred individual components are present in such extracts (Risby and Sigsby, 1980; U.S. EPA, 1980). The substances that have been found in organic solvent extracts of diesel exhaust particulates include a variety of polycyclic aromatic hydrocarbons like benzo[*a*]pyrene (Lyons, 1962; Risby and Sigsby, 1980; Santodonato *et al.*, 1978; Schuetzle *et al.*, 1980; U.S. EPA, 1980; Williams and Chock, 1979; Zweidinger *et al.*, 1980a, 1980b) and structurally related substances.

Bacterial bioassays of fractionated diesel exhaust particulate extracts indicate that mutagenic activity does not reside primarily in the polycyclic aromatic hydrocarbon fraction. This conclusion is based on the observation that the addition of enzyme preparations (S9 mix) required for the metabolic activation of polycyclic aromatic hydrocarbons leads to a decrease in mutagenic potency (Claxton and Barnes, 1980).

Consequently, it has become apparent that attention must be given to the isolation and characterization of substances that are "direct-acting" mutagens (Choudhury and Doudney, 1979; Wei et al., 1980). The situation is somewhat cloudy, however, because methylene chloride extracts of diesel exhaust particulates have been reported to inhibit the metabolic oxidation and mutagenic activity of benzo[a]pyrene as measured by the Ames test in the presence of the S9 mix (Brooks et al., 1979; Pederson, 1979). Therefore, the conclusion that diesel exhaust particulates contain mainly direct-acting mutagens probably should be viewed with caution, because the activity of mutagens requiring metabolic activation may be masked by the "enzyme inhibitors" that may be present in the extracts.

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[*] The S9 mix is the S9 fraction obtained from liver homogenates supplemented with the cofactors required for enzymatic activity.

Of the various types of direct-acting mutagens that may be present in diesel exhaust particulate extracts perhaps the most likely are arene oxides* and nitroarenes.† Some investigators have postulated that both are generated as artifacts through the epoxidizing and nitrating potential (deLamare and Ridd, 1969; Pitts, 1979) of certain gaseous components present in diesel exhaust that may chemically interact with the parent arenes during the collection process (Pitts et al., 1978b, 1979). Analogous chemical reactions also may transform arene hydrocarbons present in the atmosphere (Lane and Katz, 1977; Pitts, 1979; Pitts et al., 1978b).

Some evidence suggests that arene oxides may not contribute to the mutagenic activity of diesel exhaust particulate extracts because preincubation of these extracts with DNA does not result in significant loss of mutagenic activity (Pederson, 1979, 1980). Model arene oxides such as benzo[a]pyrene-4,5-oxide have been shown to react spontaneously with DNA. This, in turn, causes a loss of mutagenic activity (Pederson, 1979, 1980).

There is reasonable evidence, although far from conclusive, to suggest that nitroarenes may be of particular importance in the mutagenic activity of diesel exhaust particulate extracts (Pederson, 1980). Of relevance here is the observation that bacterial cell lines sensitive to the mutagenicity of diesel exhaust particulate extracts are known to contain nitroreductases that are capable of metabolizing the parent nitroarenes to nitroso and _N_-hydroxy species. These metabolites are potential alkylating agents that may react with DNA (Claxton, 1979; Pederson, 1980). Some conflicting evidence is presented in recent reports that incubations under anaerobic conditions, which are known to enhance the mutagenic activity of the potent nitroarene mutagen 2-nitrofluorene, appear to have no significant effect on the mutagenic potency of diesel exhaust particulate extracts (Lofroth, 1979; Pederson, 1979; Wei _et al._, 1980).

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* Arenes refer to aromatic hydrocarbons (e.g., benzene, naphthalene, pyrene). Arene oxides contain an oxygen atom fused to the arene nucleus via a three-membered ring.

† Nitroarenes contain one or more attached $-NO_2$ moieties. The term "direct-acting" may be misleading in the case of nitroarenes because the mutagenicity of such compounds is dependent on their bioreduction to chemically reactive intermediates that may modify DNA.

Clearly, the limited information concerning the chemical characterization of the various components in diesel exhaust frustrates attempts to interpret the mutagenic effects observed in bacterial cells. It is well known, for example, that the nitroarene, nitrofuran, is highly mutagenic in the Ames assay but is weakly or nonmutagenic in normal mammalian cells (Goodman _et al._, 1977) and in nitroreductase-deficient derivatives of a _Salmonella_ strain (TA98) (Pederson, 1979). Preliminary data suggest that diesel exhaust particulate extracts also are mutagenic in mammalian cell lines, such as human fibroblasts and _xeroderma pigmentosum_ cells (McCormick _et al._, 1979a, 1979b). However, complete understanding of any potential damage that substances contained in diesel exhaust may cause to normal human cells will require their further characterization and better understanding of the mechanisms by which the substances express their mutagenicity.

MICROBIAL STUDIES

The mutagenic activity of diesel exhaust particulate extracts has been clearly established in the Ames (_Salmonella_) reverse mutation assay (Claxton, 1979; Huisingh _et al._, 1978; Liber _et al._, 1979; Mitchell _et al._, 1979; Siak _et al._, 1979; Wei _et al._, 1980). The greatest activity was found to be localized in fractions that did not require S9 activation; _Salmonella_ strains TA98 and TA100 proved to be most sensitive (Huisingh _et al._, 1979; Siak _et al._, 1979; Wei _et al._, 1980). There is evidence, however, for some S9-induced mutagenic activity from other studies (Risby et al., 1979; Siak et al., 1979). Most of these mutagens appear to be of the frameshift type.*

Fractionation programs have been used in an attempt to define the location and identity of the specific mutagens (Huisingh et al., 1978; Lofroth, 1979). Further fractionation of the neutral fraction has revealed mutagenic activity in the oxy-1, transitional, and neutral components, with the transitional fraction containing the most active direct-acting mutagens (Huisingh et al., 1979; Schuetzle et al., 1980; U.S. EPA, 1979d).

The use of the Salmonella test as a guide in fractionation efforts has been highly developed in the research program at the Health Effects Research Laboratory of the EPA in Research Triangle Park, North Carolina. It demonstrates the utility of this test as an analytical tool as well as a bioassay method. One potential problem with this approach, however, is the distorted view of mutagenic potency given by this assay when nitroarenes are involved. The high level of nitroreductase activity in Salmonella may lead to a biased view of the importance of nitroarenes. In nitroreductase-deficient derivatives of the Salmonella strain as well as in typical mammalian cells, the activity of these mutagens is reduced considerably (Pederson, 1979). Accordingly, the presumed biological significance of direct-acting versus indirect-acting (promutagenic) agents needs to be modified.

Such nitroarenes as nitrofluorene appear to be direct-acting in the Ames test, yet are not strongly mutagenic in mammalian cell assay systems that lack high levels of nitroreductase. For this reason, it might be advisable to include nitroreductase-deficient Salmonella strains in the biodirected fractionation program. This might minimize the possibility of a bias towards fractions highly mutagenic for Salmonella but not for mammals.

The fractionation efforts being pursued at the EPA laboratory in Research Triangle Park, at General Motors Research Laboratories, and elsewhere are important because they are designed to define the mutagenic agents present in diesel exhaust particulate extracts. The results could lead to methods of eliminating or reducing the levels of chemical agents. Understanding the nature of the mutagens will also be important in making judgments concerning the probability of their being effective animal mutagens, carcinogens, or teratogens at low exposure levels.

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* Frameshift-type mutagens cause genetic changes involving a quantitative addition or deletion of DNA bases.

In addition to demonstrating that bacterial mutagens are present in diesel exhaust particulate extracts prepared with organic solvents, the Ames test has proven useful for examining other characteristics of diesel exhaust particulates. Some of the valuable information provided by these studies is reviewed below:

- The Ames test has been used at the EPA laboratory in Research Triangle Park as a method to compare extracts from a number of different sources for their mutagenic patterns (i.e., which strains respond in the presence and absence of the S9 enzyme fraction) and the apparent degree of potency exhibited when the extracts are standardized for total organic matter (Claxton, 1979). The comparison shows that diesel exhaust particulate extracts rank high on the list that includes coke-oven emissions, roofing tar, cigarette-smoke condensate, and extracts of gasoline engine exhaust particulates. (See Table 2.1.)

One difficulty with such comparisons is the variability among sources of the same class. For example, Table 3.1 provides the chemical back-up information for four diesel engines; the other samples permit a comparison of mutagenicity with the extractable organics and relative level of benzo[*a*]pyrene in the emissions. If there is a substantial degree of variation within a sampling of gasoline engine exhaust extracts, for instance, or of smoke condensate from different types of cigarettes, then it would be extremely difficult to interpret quantitative differences among groups with respect to the predicted degrees of health hazards. However, if the qualitative rank order remains unchanged, the comparisons would be more useful. This rationale is applicable to comparisons using all types of bioassays. Still, for general comparative purposes, it would be advisable to include nitroreductase-deficient *Salmonella* strains in the ranking program.

TABLE 2.1 A Comparison of the Mutagenic Activity of Extracts from Different Sources Standardized for Total Organic Matter (specific activities at 100 μg of Organic Material)[a]

	TA98		TA100	
Sample	+S9	-S9	+S9	-S9
	Diesel Engine Exhaust Extracts			
Caterpillar	59.3	65.9	115.2	167.8
Nissan	1,367.1	1,225.2	881.7	1,270.1
Oldsmobile	318.7	614.8	169.9	247.5
Volkswagen Rabbit	297.5	399.2	426.0	641.6
	Gasoline Engine Exhaust Extracts			
Mustang II	341.9	137.8	228.0	196.5
	Comparative Samples			
Cigarette-smoke condensate	98.2	Neg.	--	Neg.
Coke-oven emissions	251.6	164.1	265.6	259.4
Roofing tar	98.7	Neg.	420.0	Neg.
	Control Compound			
Benzo[a]pyrene	15,202.3[b]	NT[c]	26,438.0[a]	NT[c]

[a] A linear regression line was developed from the linear portions of the dose-response curves for positive test samples. The equation of that line was used to calculate the expected response at 100 μg of organic material. This is the specific activity.

[b] Extrapolation.

[c] Not tested.

Source: Claxton (1979).

- Studies at the EPA laboratory in Research Triangle Park (Claxton, 1979), in Sweden (Lofroth, 1979), and in Japan (Ohnishi et al., 1980), using the Ames test to compare the mutagenic activity of diesel- and gasoline-powered engine exhaust particulates, have led to generally consistent results. Diesel exhaust particulates appear to have more extractable mutagens on a mass basis than do those from gasoline engines either with or without catalytic converters. However, only the EPA's Research Triangle Park research program carried out these comparisons using other bioassays as well. This is unfortunate because, as Nesnow and Huisingh (1979) have shown, the rank order is not consistent among all of the bioassays. This is an indicator of the possible skewing toward high mutagenic activity introduced by the microbial nitroreductase activity.

- At General Motors Research Laboratories and at the Inhalation Toxicology Research Institute of the Lovelace Biomedical and Environmental Research Institute, considerable effort has been devoted to studies of the availability of mutagens from the core of the diesel exhaust particulate (Brooks _et al._, 1979; Chan _et al._, 1979; Siak _et al._, 1979). The need for these studies was based on unpublished observations in several laboratories that unextracted diesel exhaust particulates had little or no mutagenic activity in the Ames assay. The principal question, then, is the extent to which the organic mutagens are extractable (bioavailable) under normal physiological conditions.

In an attempt to evaluate the bioavailability of the substances adsorbed on diesel exhaust particulates, the particulates were suspended in various organic solvents as well as in real and simulated biological fluids. The supernatants were then evaluated for their mutagenic activity using the Ames test (see Figure 2.1, Figure 2.2, and Figure 2.3). Aqueous and saline extracts showed no mutagenic activity. Likewise, lung lavage fluid, simulated lung lavage fluid, and bovine serum albumin were not capable of extracting biologically significant levels of bacterial mutagens. Extraction with fetal calf serum did show very low but reproducible increases in the presence of direct-acting frameshift mutagenicity as shown in Figure 2.4 and Figure 2.5.

Other studies reported by King and coworkers (1980) have suggested that more of the particulate-bound chemicals than anticipated may be extracted in physiological fluids. Diesel exhaust particulates from the Nissan diesel engine were exposed to physiological fluids (serum and lung cytosol), the treated particulates from the fluid collected, and a second extraction performed with dichloromethane. Most of the mutagenic organic compounds had been removed by the physiological fluids (79 to 85 percent) and were then held in a nonmutagenic state. The mutagens could be removed from the physiological fluids by treating with XAD-2 resin;

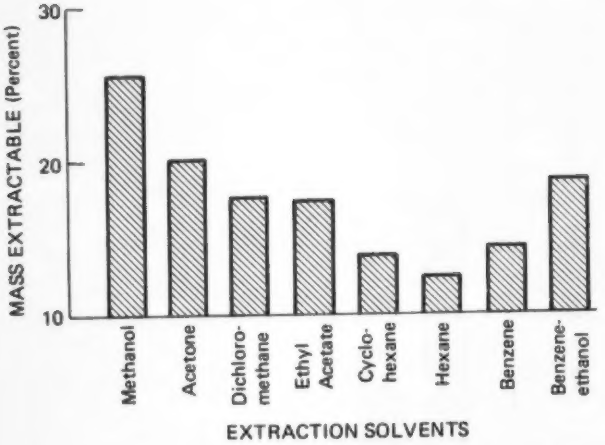

FIGURE 2.1 Percent of mass of diesel exhaust particulates extractable by organic solvents. (Source: Siak et al., 1979.)

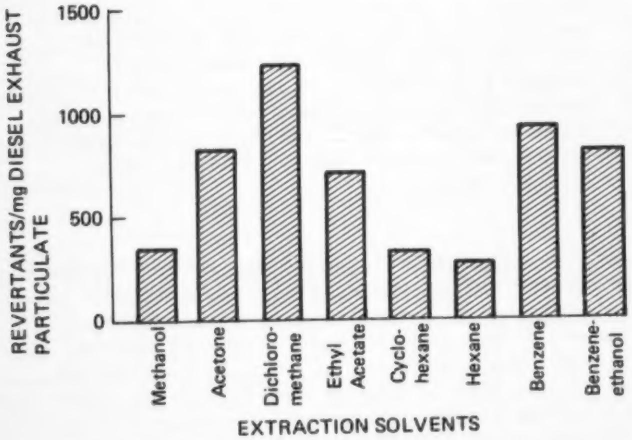

FIGURE 2.2 Mutagenic activity of diesel exhaust particulate extracts expressed as TA98 net revertants/mg of particulate without S9 activation. (Revertants were TA98 His-positive.) (Source: Siak et al., 1979.)

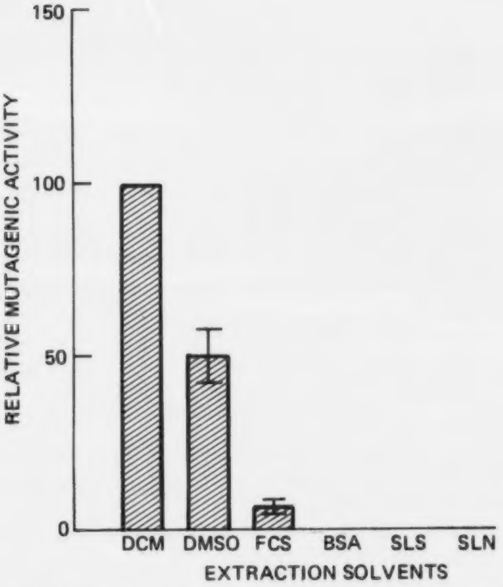

FIGURE 2.3 Comparison of the mutagenic activities of diesel exhaust particulate extracts using dichloromethane (DCM), dimethyl sulfoxide (DMSO), fetal calf serum (FCS), 0.5 percent bovine serum albumin (BSA), simulated lung surfactant (SLS), and saline (SLN). (Source: Siak _et al_., 1979.)

however, the released mutagen(s) was (were) S9-dependent in the Ames test, unlike the activity found in dichloromethane extracts of diesel exhaust particulates.

These studies require careful review because they are crucial to any analysis of _in vivo_ health hazards. Because of the high levels of benzo[_a_]pyrene known to be located in the Nissan particulate fraction, the data of King and coworkers (1980) could be said to reflect serum-mediated removal of benzo[_a_]pyrene (which is known to occur rather easily) from the particulate core. If this is so, then the mutagens still remaining adsorbed to the particulate core after treatment with physiological fluids (15 to 20 percent of the total) may be significant,

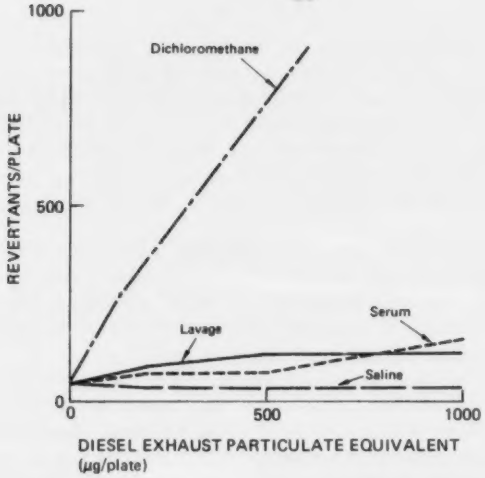

FIGURE 2.4 Dose-response relationship for mutations induced in the TA98 strain by extracts of diesel exhaust particulates with a high specific mutagenic activity. (Source: Brooks _et_ _al_., 1979.)

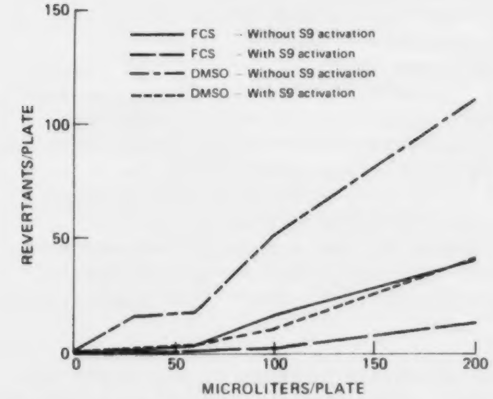

FIGURE 2.5 Dose-response curves of dimethyl sulfoxide (DMSO) and fetal calf serum (FCS) extracts of diesel exhaust particulates. (Revertants were TA98 Hispositive.) (Source: Siak _et_ _al_., 1979.)

especially when compared with other diesel exhaust particulates with much less bound benzo[a]pyrene--e.g., those in the exhaust of an Oldsmobile diesel engine have 150 times less benzo[a]pyrene than the Nissan (see Table 3.1). Accordingly, to determine the significance of these findings, diesel exhaust particulates from the Oldsmobile or Volkswagen engines should be studied in a similar manner.

The bioavailability studies suggest not only that there may be some release of organic mutagens from the carbonaceous core in the presence of body fluids, but also that very little of the mutagenic activity is expressed due to rapid binding of the molecules. The phenomena of macrophage phagocytosis, lung cell phagocytosis, and particulate processing may also be important in the ultimate bioavailability. This raises important questions, because these phenomena may well be involved to a great extent in releasing mutagens from the particulates. McCormick and coworkers (1979a), for example, show that in human fibroblast cultures nonextracted diesel exhaust particulates were located intracellularly and produced DNA-damaging cytotoxicity.

- Environmental effects might modify the mutagenic activity of the particulates to a considerable extent following their emission from the tailpipe. Ultraviolet light from the sun, atmospheric oxidation, and changes in temperature all may act to modify the chemical structure and in turn change the biological activity of the bound organics. One study by Claxton and Barnes (1980), involving the investigation of diesel exhaust particulate extracts under smog chamber conditions, suggests that the mutagenic activity of particulates for bacteria is reduced by the introduction of ozone into the system. This study indicates that ultraviolet irradiation does not significantly alter activity. Findings of this nature may well have an influence on hazard assessments. However, they should be carried further into mammalian systems before much significance is attributed to them.

The data from microbial assays, particularly the Ames Salmonella test, have provided significant information with respect to:

- The presence and chemical characterization of mutagenic substances on diesel exhaust particulates;
- The possible types of chemical entities present that have mutagenic activity;

- The availability of the mutagens when extracted with a variety of solvents, including biological fluids;
- The modification of some of these mutagenic substances by environmental factors; and
- The comparison between the mutagenic activity of diesel exhaust particulate extracts and other environmental mutagens produced during hydrocarbon combustion.

The results from most of the research programs tend to show a consistent pattern. Caution needs to be exercised, however, in directly extrapolating the bacterial results to in vitro or in vivo mammalian systems. Differences between bacterial and mammalian cells with respect to such properties as mutagen activation, cell permeability, and particulate processing indicate that significant differences in responses between bacterial and mammalian cells will be observed.

Bacterial assays are more effectively used as analytical tools, and reliance should not be placed on the bacterial data for making hazard assessments. As analytical tools, these tests can answer questions not easily studied with more elaborate bioassays. In addition, further emphasis should be placed on identifying chemical species using nitroreductase-deficient bacterial strains in conjunction with the standard strains.

The addition of other microbial systems to the research programs at the EPA laboratory in Research Triangle Park and elsewhere has added very little to the data base developed on bacteria. Diesel exhaust particulate extracts prepared with organic solvents have produced mutagenic (Loprieno et al., 1979) and recombinogenic (Loprieno et al., 1979; Mitchell et al., 1979) effects in yeasts. The responses appear both with and without S9 activation. The yeasts are less sensitive. Thus, much higher concentrations are required to produce significant effects. The data reinforce the mutagenic activity of such extracts but add very little in new information. E. coli DNA repair assays also support the presence of DNA-damaging components in these extracts, but they fail to add significant information to that obtained from Salmonella assays (Doudney et al., 1979).

IN VITRO MAMMALIAN CELLS

The data base provided by this group of studies is not extensive. Yet it may well contribute the most important information for developing *in vitro* evidence in support of a carcinogenic finding. This is especially true in the case of diesel exhaust particulates because of the high level of nitroreductase of *Salmonella* in the assay for nitroarenes. Thus, the mammalian *in vitro* tests may provide the best evidence for the types and degrees of genetic alterations caused by chemicals in diesel exhaust particulate extracts. Mammalian *in vitro* assays will answer three major questions:

- Are the gene mutagens detected in *Salmonella* and yeasts also gene mutagens in mammalian cells, and under what conditions?
- Do diesel exhaust particulate extracts or the unextracted particulates produce chromosomal abnormalities?
- Can the extractable organic chemicals transform mammalian cells to a tumorigenic state and, if so, under what conditions?

A summary of the available *in vitro* mammalian cell studies is tabulated in Table 2.2. The majority of these studies were performed on site or under contract from the EPA laboratory in Research Triangle Park and from General Motors Research Laboratories. With few exceptions there has been little duplication of these studies. Therefore, there is, as yet, no substantial confirmation of the response data.

Gene Mutations

The data for gene mutation in mammalian cells appear to be somewhat mixed. Most of the assays show some response but not always under the same conditions. The mouse lymphoma test was conducted both by the EPA laboratory in Research Triangle Park and by General Motors Research Laboratories. Both studies concluded that mutagenic activity was present under activation and nonactivation conditions (Mitchell *et al.*, 1979; Rudd, 1979). A study conducted at General Motors Research Laboratories with V-79 hamster cells proved to be negative at concentrations up to 100 μg of diesel exhaust particulate extract/ml. It is very possible that the negative response in this assay was a function of dose. A concentration of 100 μg extract/ml was not mutagenic in the mouse lymphoma assay as well; however, although higher levels of extract could be tested in the lymphoma assay

TABLE 2.2 Summary of Nine Mammalian *In Vitro* Cell Assays Performed with Diesel Exhaust Particulate Samples

Test	Endpoint Detected	Type of Sample Tested	Response	Reference
Mouse lymphoma assay	Gene mutation	OSE[a]	+(+S9/-S9)	Rudd (1979); Mitchell *et al*. (1979)
Human lymphoblast assay	Gene mutation	OSE	+(+S9)	Liber *et al*. (1979)
V-79 hamster cell assay	Gene mutation	OSE	+(+S9/-S9)[b]	Rudd (1979)
Balb/c 3T3 cell assay	Gene mutation	OSE	+(+S9/-S9)	Curren *et al*. (1979)
WI-38 human cells	UDS (DNA repair)[c]	OSE	+(+S9/-S9)	Mitchell *et al*. (1979)
EUE human cells	UDS (DNA repair)	OSE	+(+S9/-S9)	Loprieno *et al*. (1979)
Human fibroblasts (normal and *xeroderma pigmentosum*)	DNA repair	OSE	+(-S9)	McCormick *et al*. (1979a)
		Whole particulate	+(-S9)	
Balb/c 3T3 cell assay	Cell transformation	OSE	+(+S9/-S9)[b]	Curren *et al*. (1979)
Chinese hamster ovary cell line	Sister chromatid exchange	OSE	+(+S9/-S9)	Mitchell *et al*. (1979)

[a] OSE = organic solvent extract.

[b] UDS = unscheduled DNA synthesis.

[c] Only Nissan diesel exhaust particulate extract was positive; Oldsmobile and Caterpillar diesel exhaust particulates were negative.

and produce mutations, higher levels in the V-79 cells were too toxic (Rudd, 1979).

Ouabain[*] resistance was studied in Balb/c 3T3 cells by Curren and coworkers (1979). The extracts from a Nissan engine proved to be weakly mutagenic in this system as well. Oldsmobile and Caterpillar engine exhausts were not active. The responses were marginal but this may be a function of the mutant selection system, which is biased toward picking up base-pair substitution mutagens[†] rather than frameshift mutagens. Most of the evidence, however, indicates that diesel exhaust particulate mutagens are predominantly of the frameshift type.

In human lymphoblasts, Liber and coworkers (1979) showed that diesel exhaust particulate extracts are mutagenic when the system is activated by the addition of an S9 mix. This finding differed from that with the mouse lymphoma cells. However, it may be attributable to differences between the two systems in such factors as cell sensitivity, cell metabolism, or target site specificity.

It can be concluded from these studies that diesel exhaust particulate extracts contain mammalian cell mutagens. The activity of these mutagens is not nearly as high as that seen with the *Salmonella* assay. This is an indication that the bacterial nitroreductase is in fact giving a biased picture of their potency or that the bacterial mutagens and the mammalian cell mutagens are different, or possibly both. The situation arising from this uncertainty might be that the compounds producing mutations in cultured mammalian cells may be the agents responsible for cell transformation rather than the chemicals producing bacterial mutation.

At present, there is no evidence that these mammalian cell mutagens reach the germ cells of mammals *in vivo*. Thus, the risk of heritable mutation cannot be readily assumed (see following section).

Studies on the *in vitro* mutagenicity of whole particulate samples have not, as yet, been performed.

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[*] Ouabain is a glycosidic cardenolide obtained from the seeds of *Strophanthus gratus*, and used as a cardiotonic.

[†] Base-pair substitution mutagens cause a genetic change involving a qualitative substitution of DNA bases.

However, they might be important for confirmation of the bacterial responses.

Chromosome Aberrations

There is virtually no evidence that diesel exhaust particulates or their extracts either do or do not damage chromosomes *in vitro*. This is unfortunate because it strongly affects the interpretation of most of the *in vivo* studies, in which a clastogenic (chromosome breakage) endpoint was used. (See Table 2.2, summarizing *in vivo* studies.)

Without any information about whether diesel exhaust particulate extracts are capable of inducing chromosome aberrations under sensitive *in vitro* conditions, it is extremely difficult to interpret the negative *in vivo* findings. The only reasonable assumption must be that the mutagens detected in diesel exhaust particulates are not clastogenic. In fact, this may well be consistent with the evidence presented above indicating that these are largely frameshift mutagens, because such types of mutagens are highly inefficient clastogens (Hollstein *et al.*, 1979).

Mitchell and coworkers (1979) show that diesel exhaust particulate extracts induced sister chromatid exchanges in Chinese hamster ovary cells. Sister chromatid exchange is a reciprocal exchange between chromatids prior to cell division. This endpoint does not correlate well with chromosome aberration induction, but it is more predictive for point mutation induction (Carrano *et al.*, 1978). Therefore, the induction of sister chromatid exchanges cannot be presented as evidence for chromosomal damage.

Without more data, very little can be concluded about the potential of diesel exhaust particulate extracts to produce chromosomal alterations. Serious efforts should be made to fill this data gap, if such studies are not already under way. Assuming that these extracts are not clastogenic, the emphasis placed on this endpoint in the *in vivo* studies would be unjustified.

Cell Transformation

The data on cell transformation were derived from studies carried out at the EPA laboratory in Research Triangle

Park. These used Balb/c 3T3 cells of the same origin as in the work on Ouabain resistance cited earlier (Curren et al., 1979). The cell transformation assay was conducted both with and without the addition of an S9 mix. This in vitro assay is considered to be highly predictive of the in vivo carcinogenic potential (Bouck and di Mayorca, 1976; Mishra and di Mayorca, 1974).

Among the exhaust extract samples tested were three from diesel engines--the Caterpillar, the Oldsmobile, and the Nissan. Of these, only the Nissan sample induced cell transformation (Curren et al., 1979). A comparison of the results is shown in Table 2.3 and Table 2.4. The activity of the Nissan engine extract is not surprising since it was found to have a much higher benzo[a]pyrene content (see Table 3.1) than the exhaust extracts of the other two engines. This compound is an efficient transforming agent for Balb/c 3T3 cells (as shown in Table 2.4).

The observation that the Oldsmobile and Caterpillar extracts failed to transform mammalian cells in vitro makes questionable whether particulate extract components (other than benzo[a]pyrene) are capable of inducing cell transformation. This may be even more important in light of the observation that the gasoline engine exhaust extract (Mustang II) was a potent inducer of cell transformation (Curren et al., 1979). Clearly, additional studies are needed to establish the transforming capacity of diesel and gasoline engine exhaust particulate extracts.

The extracts from several diesel and gasoline engine exhausts should be compared. An analysis should be done to determine if the transforming ability of the extracts from either source is a function of their benzo[a]pyrene content. If so, then a critical evaluation must be made of the possibility that the direct-acting bacterial mutagens, which predominate in the diesel exhaust particulate extracts, are not capable of transforming mammalian cells and are, therefore, noncarcinogenic. This analysis may be of crucial importance in further attempts to extrapoate in vitro mutagenesis as a predictor of in vivo carcinogenesis. Such studies should be given high priority.

Direct DNA Damage

Other studies involving mammalian cell assays have been performed. Diesel exhaust particulate extracts were

TABLE 2.3 Total Genotoxic Events Occurring After Exposure to Various Emission Extracts in the Absence of Exogenous Metabolic Activation[a]

Source Emission Extract or Type of Control	Transformation		Mutation	
	Type III Foci	TF[b] $(\times 10^{-5})^b$	OUA[c] Colonies	MF[d] $(\times 10^{-6})^d$
Caterpillar diesel engine	0	<0.68	1	0.18
Oldsmobile diesel engine	1	0.33	2	0.53
Roofing tar	2	0.61	20	3.14[e]
Nissan diesel engine	14	3.43[e]	5	1.06[e]
Coke-oven emissions	6	2.13[e]	45	8.27[e]
Mustang II gasoline engine	11	4.10[e]	27	4.49[e]
Positive control (1 μg N-methyl-N'-nitro-N-nitrosoguanidine/ml)	29	12.34[e]	139	35.50[e]
Negative control (0.25% solvent)	0	<0.34	1	0.18

[a] See Curren et al. (1979) for the methods in data transformation used to obtain the rankings shown in this table.

[b] TF = Transformation frequency for Type III foci only.

[c] Ouabain-resistant.

[d] MF = Mutation frequency.

[e] Data significantly different from combined negative controls at $p < 0.05$.

Source: Curren et al. (1979).

TABLE 2.4 Total Genotoxic Events Occurring After Exposure to Various Emission Extracts in the Presence of Exogenous Metabolic Activation[a]

Source Emission Extract or Type of Control	Transformation		Mutation	
	Type III Foci	TF $(\times 10^{-5})$[b]	OUA[c] Colonies	MF $(\times 10^{-6})$[d]
Caterpillar diesel engine	0	<0.30	1	0.20
Oldsmobile diesel engine	0	<0.55	2	0.49
Roofing tar	4	1.07	11	1.73[e]
Nissan diesel engine	3	1.04	10	1.81[e]
Coke-oven emissions	6	2.41[e]	--	--
Mustang II gasoline engine	7	2.92[e]	19	3.97[d]
Positive control (12.5 μg benzo[*a*]pyrene/ml)	19	10.30[e]	48	14.20[d]
Negative control (0.25 percent solvent)	0	<0.36	1	0.26

[a] See Curren *et al.* (1979) for the methods in data transformation used to obtain the rankings shown in this table.

[b] TF = Transformation frequency for Type III foci only.

[c] Ouabain-resistant.

[d] MF = Mutation frequency.

[e] Data significantly different from combined negative controls at $p < 0.05$.

Source: Curren *et al.* (1979).

evaluated in two independent assays measuring stimulation of the DNA repair enzyme system (unscheduled DNA synthesis). Both used human fibroblast cells--one, WI-38 cells (U.S. EPA, 1979d) and the other EUE cells (Loprieno *et al.*, 1979). In both cases, the extracts did not produce significant effects. The results cannot be considered highly important because both assays employed the scintillation method of analysis, which is much less sensitive than the more time-consuming autoradiographic method (Williams, 1977). In fact, few laboratories still employ the methods used in these evaluations.

Another series of studies that relied upon a completely different approach produced some interesting findings. McCormick and coworkers (1979a, 1979b) investigated the differential cytotoxicity between normal and *xeroderma pigmentosum* fibroblasts as an indicator for DNA-damaging agents. Populations of both cell types should show the same death curves when treated with non-DNA-damaging toxicants. However, chemicals with specific DNA-damaging properties should show greater toxic effects in the *xeroderma pigmentosum* cells, because they lack a complete DNA repair capacity (Cleaver and Bootsma, 1975; Setlow, 1978). Results of the evaluation indicate that both whole unextracted diesel exhaust particulates, as well as the extracts, produced greater cytotoxicity in the *xeroderma pigmentosum* cells than in normal cells. This suggests that the phagocytosis of the whole particulates by the cells may result in the intracellular release of some of the bound biologically active organic molecules.

In these studies, particulates were found within the cells. It should be kept in mind that this technique is extremely sensitive and the type of damage detected is not direct evidence for mutagenicity (McCormick *et al.*, 1979a). However, it is one of the few pieces of information that can be directly applied to answering the question about bioavailability. (See Table 3.9 and page 78.)

In summary, the panel concludes that to a reasonable extent the *in vitro* mammalian cell assays support the microbial data in that diesel exhaust particulate extracts produce positive responses. There is need for additional work in this area before the full value of these techniques can be realized. The following conclusions may be made on the basis of currently available evidence:

- Diesel exhaust particulate extracts contain chemicals capable of inducing gene mutation in mammalian cells.

- It is not known whether these extracts also contain clastogens (substances that damage chromosomes). More studies are needed to make this determination.
- The ability of diesel exhaust particulate extracts to induce cell transformation *in vitro* is questionable. The extracts from Oldsmobile and Caterpillar engine exhausts were not active. The one active sample (from the Nissan engine exhaust) had an unusually high content of benzo[*a*]pyrene, which clouds the issue, because this component alone may account for the response. More studies are needed in this area.
- The studies of McCormick and coworkers (1979a, 1979b) suggest that mammalian cells are capable of extracting biologically active amounts of organic compounds from diesel exhaust particulates intracellularly. This is a potentially significant finding which suggests that materials adsorbed on diesel exhaust particulates may be bioavailable, at least to a limited degree.

COMPARATIVE ANALYSES DERIVED FROM SHORT-TERM ASSAYS

Before proceeding to a consideration of the *in vivo* studies, it will be valuable to estimate how the microbial and mammalian cells respond to a series of related samples. The results that are presented in Table 2.5 and Table 2.6 are a composite of the available data from all sources, although most were generated by the EPA laboratory in Research Triangle Park. Other than some test samples requiring the addition of an S9 mix, Tables 2.5 and 2.6, which include samples of emission extracts and condensate, do not show any type of consistent pattern either between the microbial and mammalian cell data or within the mammalian cell data. In addition, there is no clear comparative trend between the results of any of the short-term assays and the *in vivo* skin tumor-initiation response as shown in Tables 2.5 and 2.6. It needs to be emphasized that meaningful analysis would require independent reproducibility of responses and a wider range of test samples (e.g., several gasoline engine exhaust extracts).

One potentially important point is shown in Table 2.7, in which the relative ranking for four diesel engines and one gasoline engine are given for the Ames test and the mouse lymphoma assay. Specifically, it appears that the microbial systems are more responsive to the diesel than to the gasoline engine exhaust particulate extracts, and the mammalian cell test appears to respond better to the gasoline engine exhaust extract. It is known that exhausts from the two engine types have different chemical compositions. Possibly, the diesel exhaust particulate extracts contain more nitroaromatics and hydrocarbons.

With respect to predicting carcinogenicity, the data from both microbial and mammalian cell assays support a presumption of carcinogenic activity for extracts of diesel exhaust particulates. However, absolute activity per unit dose does not permit a definitive assessment of their potency. The microbial data, nevertheless, could be interpreted as predicting potent carcinogenic activity for diesel exhaust extracts, whereas the mammalian cell responses could be interpreted as indicating a much weaker carcinogenic activity.

IN VIVO STUDIES

The *in vivo* assays conducted to study genetic effects are described in Table 2.8. The array of tests shown can be characterized as oriented toward the detection of chromosomal damage (aberrations and micronuclei) rather than for genetically undefined endpoints (sister chromatid exchange and spermhead abnormalities). Another characteristic of these studies is that the primary route of administration is by inhalation. The only test in Table 2.8 with a proven capability of responding directly to gene mutagens is the *Drosophila* sex-linked recessive lethal assay.

The studies in which clastogenic effects were evaluated were all negative with the exception of a micronucleus test conducted on Chinese hamsters (Pereira *et al.*, 1979a). The results of this test suggest a weak response, but other evidence for micronuclei induction (based on results from mice and dogs) does not support these results. The increase over the background was only 50 percent, which is somewhat questionable. Chromosome aberration studies in mice and Chinese hamsters likewise do not support the evidence for diesel exhaust particulate-induced chromosome breakage. The aberration analyses should be somewhat more sensitive and give better resolution. This suggests that the slight increase reported in the micronucleus study was spurious.

TABLE 2.5 Relative Activity Ranking for Eight Samples in Four Short-term Assays and in *In Vivo* Skin-tumor Initiation[a] Under Nonactivation Test Conditions (lowest number = highest relative activity)

Sample	Ames	Mouse Lymphoma	Sister Chromatid Exchange	Cell Trans-formation	Skin-tumor Initiation
Gasoline engine (Mustang II)	5	3	6	1	5
Coke-oven emission	4	2	1	3	1
Cigarette-smoke condensate	Not active	4	3	Not tested	Negative
Roofing tar emissions	Not active	8	4	Negative	3
Diesel engine (Nissan)	1	5	2	2	2

Diesel engine (Oldsmobile)	2	7	Not tested	Negative	4
Diesel engine (Caterpillar)	6	6	7	4	Negative
Diesel engine (Volkswagen)	3	1	6	Not tested	Not tested

[a] Ames strain TA98 data adjusted to revertants/mg organics. Mouse lymphoma data adjusted to revertants/10^6 survivors at the LD_{50} concentration. Sister chromatid exchange data adjusted to exchanges per cell at a constant dose level. Cell transformation data not adjusted. These data are similar but not identical to rankings developed by Nesnow and Huisingh (1979); however, the similarities are close enough to reach similar conclusions.

Source: Developed from: Claxton 1979, Curren et al. (1979), Mitchell et al. (1979), and Slaga et al. (1979).

TABLE 2.6 Relative Activity Ranking for Eight Samples in Four Short-term Assays and in *In Vivo* Skin-tumor Initiation[a] Under Activation Test Conditions (lowest number = highest relative activity)

Sample	Ames	Mouse Lymphoma	Sister Chromatid Exchange	Cell Transformation	Skin-tumor Initiation
Gasoline engine (Mustang II)	2	1	Not tested	1	5
Coke-oven emission	5	3	2	2	1
Cigarette-smoke condensate	7	7	Not tested	Not tested	Negative

Roofing tar emissions	6	5	1	3	3
Diesel engine (Nissan)	1	6	3	4	2
Diesel engine (Oldsmobile)	3	4	Not tested	Negative	4
Diesel engine (Caterpillar)	8	8	Not tested	Negative	Negative
Diesel engine (Volkswagen)	4	2	4	Not tested	Not tested

[a] Ames strain TA98 data adjusted to revertants/mg organics. Mouse lymphoma data adjusted to revertants/10^6 survivors at the LD_{50} concentration. Sister chromatid exchange data adjusted to exchanges per cell at a constant dose level. Cell transformation data not adjusted. These data are similar but not identical to rankings developed by Nesnow and Huisingh (1979); however, the similarities are close enough to reach similar conclusions.

Source: Developed from: Claxton (1979), Curren *et al.* (1979), Mitchell *et al.* (1979), and Slaga *et al.* (1979).

TABLE 2.7 Relative Activity Ranking of Automobile Engine Exhaust Extract Samples in the Ames and Mouse Lymphoma Gene Mutation Assays[a]

Engine	AMES (TA98)		Mouse Lymphoma	
	+S9	-S9	+S9	S9
Diesel (Nissan)	1	1	4	3
Diesel (Oldsmobile)	3	2	3	5
Diesel (Caterpillar)	5	5	5	4
Diesel (Volkswagen)	4	3	2	1
Gasoline engine (Mustang II)	2	4	1	2

[a] See Table 2.5 for explanation of ranking.

Source: Developed from: Claxton (1979), Curren *et al.* (1979), and Mitchell *et al.* (1979).

The absence of clastogenic effects is not surprising because the types of mutagenic agents associated with particulate extracts would not be anticipated to be strongly clastogenic. Therefore, it would appear to be difficult to justify the tremendous effort expended to search for evidence of chromosomal breakage. Unfortunately, no *in vitro* studies for clastogenicity were conducted, making the presumption of nonclastogenicity less convincing. Positive *in vitro* data might put the *in vivo* results into a different perspective. If *in vitro* results were clearly negative, lack of *in vivo* activity could be considered consistent with the absence of inherent clastogenicity. Conversely, positive *in vitro* test results might be interpreted to mean that emphasis on conducting *in vivo* studies with greater sensitivity should be pursued.

The *in vivo* assays measuring genetically undefined effects (*i.e.*, endpoints without established mechanisms of induction or specificities for either gene or chromosomal damage) were also negative. An indication of a positive response in spermhead abnormalities was suggested in Chinese hamsters by the same investigators who reported the increase in micronuclei (Pereira *et al*., 1979a).

Without confirming data from the other spermhead abnormalities studies, the weak response in Chinese hamsters must be viewed with caution. The data for sister chromatid exchange were negative. It might be of value, however, to extend and amplify these studies, particularly in the lung cell evaluation, for two reasons:

- The test is sensitive and can be performed *in vivo*; and
- *In vitro* data show that diesel exhaust particulate extracts can induce this change.

Based on *in vitro* concentration estimates, it is most likely that the level of mutagenic substances released from inhaled particulates was too low to reach the minimal effective concentration for inducing sister chromatid exchange under the conditions reported.

The use of the *Drosophila* sex-linked recessive lethal assay was not an appropriate model for the following reasons:

- The structure and physiology of the respiratory systems of insects and mammals differ markedly;
- These differences could significantly influence the uptake and distribution of particulates into the insect respiratory system both qualitatively and quantitatively, as compared with mammals;
- The exposure levels cannot be reliably ascertained because *Drosophila* are known to be reasonably efficient at avoiding particulate matter suspended in the feeding solution; and
- The polycyclic and aromatic classes of chemical mutagens are likely to be missed in the *Drosophila* sex-linked recessive lethal assay (Fahmy and Fahmy, 1972).

Therefore, the interpretation of negative responses in this assay is of little value in the overall assessment of diesel exhaust mutagenicity.

The mammalian *in vivo* studies provide very little direction with respect to decisions concerning genetic hazard from exposure to diesel exhaust. The majority of the tests conducted were oriented toward detecting an endpoint (clastogenicity) for which there was no *in vivo* data indicating its induction by the substances

TABLE 2.8 *In Vivo* Studies Conducted to Evaluate the Genetic Effect of Diesel Exhaust

Name of Assay	Type of Endpoint Detected	Type of Sample[a]	Route of Administration	Response	Reference
Drosophila (sex-linked recessive lethal)	Gene mutation	WDE	Inhalation	Negative	Schuler and Neimeier (1979)
Drosophila (sex-linked recessive lethal)		Particulate extract	Feeding	Negative	U.S. EPA (1979d)
Abnormal spermhead:					
Mice	Unknown	WDE	Inhalation	Negative	Pereira *et al*. (1979b)
Cat		WDE	Inhalation	Negative	Pereira *et al*. (1979a)
Chinese Hamster		WDE	Inhalation	Negative	Pereira *et al*. (1979a)
Sister chromatid exchange:					
Chinese hamster bone marrow	Chromosomal change	WDE	Inhalation	Negative	Pereira *et al*. (1979a)

Dog peripheral lymphocytes		WDE	Inhalation	Negative	Benz and Belz (1979)
Syrian hamster lung cells		WDE	Inhalation	Negative	Guerrero *et al*. (1979)
Chromosome aberrations assay:	Clasto-genicity				
Chinese hamster bone marrow		WDE	Inhalation	Negative	Pereira *et al*. (1979a)
Mouse bone marrow		WDE	Inhalation	Negative	Pereira *et al*. (1979c)
Micronucleus Test:	Clasto-genicity				
Dog peripheral lymphocytes		WDE	Inhalation	Negative	Benz and Beltz (1979)
Chinese hamster bone marrow		WDE	Inhalation	Positive	Pereira *et al*. (1979a)
Mouse bone marrow		WDE	Inhalation	Negative	Pereira *et al*. (1979c)

[a] WDE = whole diesel exhaust.

Note: All samples studied were whole diesel exhausts with the exception of the U.S. EPA (1979d) study with *Drosophila*, which used diesel exhaust particulate extracts.

extracted from diesel exhaust particulates. The *Drosophila* sex-linked recessive lethal assay, the only *in vivo* test that is known to detect gene mutation induced by such extracts, is one characterized by difficulties with exposure assessment and nonresponsiveness to hydrocarbon and aromatic amine mutagens. Evaluations of sister chromatid exchange studies may be able to provide some useful *in vivo* data, but the results from preliminary studies are not encouraging.

Considering these limitations, the following conclusions may be drawn from the tests listed in Table 2.8:

- There is no convincing evidence that shows the induction of genetic alterations *in vivo* following exposure to diesel exhaust particulates. The absence of effects holds for both somatic and germ cells.
- Most currently available *in vivo* assays designed for genetic hazard assessment are oriented toward the detection of clastogenic effects, which may be the least likely to be produced by diesel exhaust particulate mutagens.
- Without additional biochemical evidence, the results summarized in Table 2.8 indicate that no significant levels of mutagens are released in exposed cells under *in vivo* conditions.

These conclusions appear to be contradictory to those initially developed from the short-term *in vitro* studies. The complicating factor appears to be the bioavailability of the mutagens bound to the particulate core. Based on results obtained with actual and simulated biological fluids, it may be that very little of the organic matter is released from the particulate *in vivo*. Because the resolving power of the *in vivo* tests is not sufficiently strong to measure extremely weak responses, it is unlikely that additional *in vivo* testing will solve these problems.

Further hazard analysis for heritable genetic effects will probably require the development of methods capable of detecting *in situ* interaction between the organic chemicals extracted from the particulates and DNA, or through further development of sensitive cellular monitors (e.g., the induction of chromosomal changes in spermatocytes).

DATA GAPS AND EVALUATION OF PROPOSED NEW STUDIES

The evidence for genotoxic activity of diesel exhaust, and hence possible carcinogenic activity is clearly demonstrated in the results obtained with short-term _in vitro_ studies. Data are needed in the following areas:

- The chemical nature of the direct-acting mutagens in the diesel exhaust particulate extracts and their potency relationships in bacterial and mammalian cells;
- The bioavailability of mutagenic and carcinogenic substances from inhaled or ingested diesel exhaust particulates;
- The transport of particulates or "biologically significant" levels of released mutagens to critical sites, such as DNA in somatic or germinal cells, because evidence for their direct interaction with DNA in covalent binding would be most useful; and
- _In vitro_ studies for clastogenicity.

Numerous proposals have been submitted from which the panel received no data. Unfortunately, none addresses any of the three data gaps perceived to be most critical. Table 2.9 lists the proposed programs and identifies the value of data that might be derived from each of the research programs. Only one or two of the proposed studies might contribute data that could be useful in hazard assessment. The others either duplicate existing studies or are not appropriate.

GENERAL CONCLUSIONS FROM SHORT-TERM AND _IN VIVO_ GENETIC STUDIES

- Mutagenic compounds, both direct-acting and S9-activated, adhere to the central carbonaceous core of diesel exhaust particulates.
- Biologically active amounts of these substances may be released from particulates that are inhaled or ingested. However, based on available evidence, whole diesel exhaust does not appear to be mutagenic in mammals.
- Studies involving _in vitro_ mammalian cell systems indicate that particulates in the exhaust of some diesel engines may contain sufficient levels of

TABLE 2.9 List of Proposed Genetic Research Programs Directed Toward Diesel Exhaust Health Effects

Proposal Title	Importance of Data Likely to be Developed	Investigating Organization
Carcinogen activation and screening in various cells	Minimal value in developing reliable hazard assessment	University of California, Berkeley
Mutagenic potential of petroleum coke and coke processing derivatives	Minimal value except for *in vitro* comparative purposes	Hazelton Laboratories
Development and application of microbial mutagenicity bioassays as a bioanalytical tool in the evaluation of complex mixtures resulting from mobile and other sources	No value to current program since the value of this test as a bioanalytical tool has already been established	EPA
Application of a confirmatory multitest battery of short-term mutagenesis and carcinogenesis bioassays to evaluate diesel vehicle exhausts in comparison with other combustion emissions	Of some value--a comparative data base in a multi-test program would be useful for filling data gaps and for extrapolation purposes	EPA

Mutagenicity of diesel exhaust in hamster lung	Data might be of significant value. This type of analysis is one of the few that might shed light on the bioavailability of genotoxic agents under *in vivo* test conditions	EPA
Tests for heritable effects induced by diesel exhaust in the mouse	Minimal value in developing a more reliable hazard assessment. Most of the proposed tests employ clastogenesis as the endpoint	EPA
Cytogenetic studies of lung cells from animals exposed *in vivo* to diesel exhausts	Data might be of significant value. This method will provide input related to risk analysis	EPA

biologically available carcinogens to produce cell transformation under conditions of high exposure.

- In limited comparisons with extracts from exhaust particulates collected from gasoline engines, diesel exhaust particulate extracts appear to contain more direct-acting bacterial mutagens. However, the activity relationships may not hold in the case of mammalian cell mutation.

3 CARCINOGENESIS

The combustion of organic matter can result in the formation of trace amounts of carcinogenic substances. Many of these belong to the class of compounds known as polycyclic aromatic hydrocarbons (National Research Council, 1972). The formation of these carcinogenic products of pyrolysis depends on such factors as the nature and composition of the materials burned and the combustion temperature. The first evidence of the presence of carcinogenic substances in the exhausts of gasoline as well as diesel engines was reported about 25 years ago (Kotin et al., 1954b, 1955). Most of the carcinogens appear to reside in the particulate rather than in the gas phase of the exhausts. However, only a few studies have been carried out on gas-phase materials alone (Cadle et al., 1979; Hare and Baines, 1979).

The particles emitted in diesel exhaust are of respirable size and most are in the submicron range (Soderholm, 1979; Vuk et al., 1979). Consequently, they can remain suspended in the air for considerable periods of time, and can be deposited in the smallest airways of the lungs. The major question is whether such air contaminants emitted by diesel engines increase the risk of developing lung cancer in exposed populations.

The respiratory tract need not necessarily be the only organ affected by inhaled particulates. Following deposition in the airways, the particulates (or materials associated with them) can reach other parts of the body via the bloodstream and the lymphatics, as well as by mucociliary clearance to the digestive tract. Lung cancer is not the only potential health hazard that might be associated with exposure to diesel engine exhausts. Chronic obstructive pulmonary disease may also result from such exposure. (See page 104 et seq.)

By the end of the century, according to the General Motors Corporation, there will be a marked increase in the number of new vehicles equipped with diesel engines entering the passenger car fleet in the U.S. Questions have arisen as to whether the displacement of gasoline engines with catalytic converters by diesel engines will significantly increase the amounts of carcinogenic contaminants in the environment (or in specific regions in the environment), and whether such an increase, should it occur, is likely to result in increased cancer incidence rates in the U.S. population or in any subpopulation thereof. In other words, a most important question is whether diesel engine exhaust, per vehicle-mile traveled, contributes substantially more carcinogenic material to the environment than does gasoline engine exhaust. Another important question is whether diesel engine exhaust acts synergistically with existing carcinogenic agents to which man is exposed.

Furthermore, it is essential to establish which variables determine the chemical nature and generation of carcinogens in diesel exhaust (fuel composition, engine operation, etc.); what measures can be introduced to reduce the emission of carcinogens from diesel engines (e.g., by the use of trap oxidizers); and what are the degree of transport, the degree of transformation, and the half-lives of diesel exhaust carcinogens in the environment.

It is generally accepted that the principal events leading to neoplastic transformation and tumor formation are common throughout the animal kingdom. This is the rationale that forms the basis for all bioassays conducted with animals, animal tissues, or cells for the purpose of determining the carcinogenicity of environmental agents. In most cases, the purpose of such studies is to determine whether the agent is likely to be carcinogenic in humans. A decisive factor in interpreting data obtained with such tests is the recognition that there are great variations among species, strains, and even individuals in the susceptibility to a given carcinogen. Even within an organism, the susceptibility of one tissue to the carcinogenicity of a given substance is different from that of others. For this reason, it is generally recommended that tests be carried out in two or more species and in more than one bioassay system.

There are many complications involved in extrapolating data obtained from laboratory animal species to man. Relevant considerations have been documented in various publications (Goldsmith and Friberg, 1977; Interagency Regulatory Liaison Group, 1979; Kotin and Falk, 1963). The panel supports the concept that transformation studies carried out with laboratory animal tissues, if conducted in scientifically and technically sound ways, are a necessary part of human health risk assessments of carcinogenicity.

Such studies can provide only indirect evidence for human carcinogenicity of the substances tested. Unequivocal evidence, obtained by laboratory tests, of the carcinogenic activity of an agent present in man's environment should be regarded as a serious signal that a potential human health hazard exists. The basis for quantitative risk assessments founded on studies with animals or animal tissues is much more complex and uncertain than that for qualitative assessments.

Emissions from combustion engines are complex physico-chemical mixtures of gases, aerosols, and particulates. This complexity necessitates that engine exhausts and materials derived from them be assayed in a variety of test systems. There are four major types of materials:

- Whole engine exhaust;
- Whole particulate fractions;
- Extracts of the particulate fractions; and
- Gaseous fractions. (These have not been investigated thoroughly. Furthermore, fractionations result in artifacts because of chemical interactions.)

In view of the projected increase in the number of light-duty vehicles powered by diesel engines, the most important questions that studies concerned with the carcinogenicity of diesel exhaust can address are:

- Does diesel engine exhaust contain carcinogenic or cocarcinogenic substances--i.e., do recent studies support previous observations that diesel exhaust contains carcinogens?
- What is the chemical nature of the major carcinogens, tumor initiators, and/or cocarcinogens in diesel exhaust?
- What is the relative carcinogenicity of diesel engine exhaust compared with gasoline engine exhaust?

- What are the most important factors controlling the formation of carcinogens in diesel engine exhaust?
- Are diesel engine exhaust materials carcinogenic for respiratory tract tissues and/or other organs?

CHOICE OF BIOASSAYS

In determining environmental cancer hazards, bioassays are used to obtain information concerning three major questions:

- Is the substance or the mixture carcinogenic?
- Is it a weak or strong carcinogen?
- What is the spectrum of target tissues likely to be, considering human exposure patterns?

The type of bioassay selected largely depends on which one of the three questions is to be answered.

- To obtain qualitative information about whether or not a material has carcinogenic activity, a number of *in vitro* cell transformation tests and *in vivo* tests can be used. In the case of combustion engine exhausts, the materials to be tested are the whole exhaust, the particulates, the extracts of the particulates, and the fractions thereof.

The advantages of *in vitro* tests are that they are usually rapid and inexpensive, and their results can be obtained in a matter of weeks. Their drawback is that they can yield false negatives (and also, though less often, false positives). This is particularly so when ill-defined, complex mixtures are to be tested. At present, these tests are considered to be highly useful primarily as screening assays.

A great variety of *in vivo* carcinogenesis assays using various routes of administration (intramuscular, intragastric, intraperitoneal, epicutaneous, etc.) are available. Some are more standardized than others (Interagency Regulatory Liaison Group, 1979). Of these, tests such as the pulmonary adenoma assay in Strain A mice (Shimkin and Stoner, 1975) or the mouse hepatoma assay (Tomatis *et al*., 1973; Ward *et al*., 1979) take advantage of the fact that organs with a relatively high "spontaneous" tumor incidence are highly sensitive to carcinogen exposure. Such exposure increases the spontaneous tumor incidence. Data can usually be obtained within 4 to 6 months as compared with 18 to 24 months in most other bioassays. This makes these assays very attractive. However, it is still uncertain whether they are measuring transforming activity or cocarcinogenic activity. Therefore, they are presently used mostly for screening.

- A number of experimental test systems can be used for determining relative carcinogenic activity and for developing reproducible dose-response curves. Among these are *in vitro* cell transformation systems using certain cells and cell colonies (e.g., 3T3 and 10T 1/2 cell colonies, and Syrian hamster embryo cells). Even though the end points usually measured in these tests are morphological changes in the cells or cell colonies (morphological transformations), they have been shown to correlate closely with the induction of neoplastic cells.

By incorporating the demonstration of neoplastic cell transformation into these *in vitro* test systems, they can be used to measure and quantitate carcinogenic activity, particularly of pure compounds. This is done by inoculating morphologically transformed cells or their offspring into compatible host animals, and determining the oncogenicity of the cell inoculum (International Agency for Research on Cancer, 1977).

Among the *in vivo* systems, the mouse skin carcinogenesis assay is the most quantitative and reproducible assay for carcinogenic polycyclic aromatic hydrocarbons (e.g., benzo[*a*]pyrene), *N*-heterocyclic compounds (e.g., dibenzacridines), and for certain alkylating agents (e.g., β-propiolactones). However, the mouse skin assay is not applicable for testing unextracted particulates. The pulmonary adenoma assay has also been shown to yield quantitative and reproducible dose responses for a fairly wide spectrum of carcinogens (Shimkin and Stoner, 1975). It should be stressed that no single carcinogenesis bioassay that has a specific organ as its major target (skin, liver, lung, etc.) is responsive to all classes of carcinogens. Consequently, for test mixtures of unknown or unidentified composition, it is necessary to use several bioassay systems. In the case of combustion engine exhausts, exhaust particulate extracts and their fractions are the most appropriate materials to be used for establishing dose-response relationships.

- To determine the likely site of the tumor response in humans who are exposed to an environmental carcinogen, it is necessary to attempt a simulation of human exposure conditions, or at least certain aspects of them, such as the route of exposure. Usually, such experiments are carried out only when there is reason to believe that the material or compound has carcinogenic activity. In the case of combustion engine exhausts, the materials to be tested are the whole engine exhaust, the exhaust particulates, and their fractional extracts.

Among the various experimental approaches, the one most closely resembling human exposure to air pollutants usually involves the exposure of rodents (e.g., rats or hamsters) by inhalation. However, experience has shown that this type of assay is often relatively insensitive (International Union Against Cancer, 1976; Laskin and Sellakumar, 1974). Marked differences exist at all levels of biological organization (anatomical, physiological, histological, and biochemical) between the respiratory tracts of humans and rodents. The inhalation assay is also the most complex of the bioassays. A commonly used approach, which in many ways represents a compromise solution, is the intratracheal instillation assay. (For review and discussion, see Nettesheim and Griesemer, 1978.) The test materials (solids or liquids) are deposited in the lungs of rodents through instillation into the trachea. Although the exposure is nonphysiological, it permits the instillation of large quantities of test materials into the lungs. It has been used extensively with a variety of carcinogens, especially polycyclic aromatic hydrocarbons in crystalline form or adsorbed on carrier dust. The tumor response elicited by this method resembles, in many respects, that seen in humans exposed to carcinogenic air contaminants, with regard to topography of tumor response and histopathological features of the preneoplastic and neoplastic lesions.

EARLY STUDIES ON DIESEL ENGINE EXHAUST MATERIALS

As early as 1936, J. Argyll Campbell reported experiments on the chronic inhalation exposure of mice to exhaust from internal combustion engines "...in concentrations somewhat resembling those obtained in traffic blocks and garages. There were no marked effects upon the well-being of the mice." No increase in lung tumor incidence was observed.

Approximately 25 years ago, Kotin and coworkers (1954a, 1955) demonstrated that the particulate phases of both gasoline and diesel engine exhausts contained polycyclic aromatic hydrocarbons, including benzo[_a_]pyrene, and that benzene extracts of the particulates in the exhausts, when applied to mouse skin, induced papillomas and carcinomas. Researchers at the same facility also produced evidence that the polycyclic aromatic hydrocarbons were eluted from the engine exhaust particulates by body fluids (Falk _et al._, 1958). In addition, they showed that the toxicity and carcinogenic potential of the emissions varied with the conditions under which the engines operated (Kotin _et al._, 1954b).

Subsequent studies by Mittler and Nicholson (1957), Wynder and Hoffmann (1962), and Hoffmann _et al._ (1965) essentially confirmed and extended the earlier findings. They established a dose-response effect with varying concentrations of emission extracts on mouse skin.

The study carried out by the General Motors Research Laboratories and the Sloan Kettering Institute for Cancer Research (Wynder and Hoffmann, 1962) compared the carcinogenic potential on mouse skin of suspensions of cigarette "tar" with that of the organic extract of gasoline engine exhaust particulates. On a gram for gram basis, the tumorigenic activity exhibited by the particulate matter of the exhaust from a 1958 gasoline engine (without a catalytic converter) was about twice that of cigarette-smoke condensate. In a later study, a 50 percent suspension of the organic matter residue of exhaust from a 1972 diesel engine induced skin tumors in 3 out of 50 mice after 15 months of application (Begeman and Klimisch, 1977).

Stupfel and coworkers (1973) reported on a chronic inhalation study in which rats were exposed to gasoline engine exhaust. They described an increased incidence of tumors (all sites) in exposed rats (8/28 versus 3/37 in controls), but stated that there was no increase in lung tumors in the exposed groups. Because of the small number of animals involved, the significance of this finding is questionable, particularly since the tumors appear in different organ sites.

Subsequently, several studies were conducted with various types of auto exhaust materials. Many of these were reported and discussed at a symposium held by the

International Agency for Research on Cancer (1977) on air pollution and cancer causes in man. All the reported studies showed carcinogenic activity for the auto exhaust materials, further confirming earlier observations (Grimmer and Bohnke, 1978).

Two other inhalation studies with whole auto exhaust were reported. One was carried out by the EPA using beagle dogs (Hyde *et al*., 1978). The dogs were exposed 16 hours per day for 68 months to unextracted and photochemically reacted auto exhaust (and other polluted atmospheres). They were sacrificed after an additional 36 months. No preneoplastic or neoplastic lesions were reported. Only hyperplastic and metaplastic lesions were observed, which are nonspecific tissue reactions not necessarily related to carcinogenesis. Moreover, cellular atypia, a feature commonly considered to be indicative of preneoplastic changes, was absent.

The purpose of the other study (U.S. DHEW, 1978) was to investigate the health effects of uranium ore mine pollutants in hamsters. One group of animals was exposed for life to the exhaust of a 3-cylinder, 43 brake horsepower, diesel engine (102 hamsters, 6 hours per day, 5 days per week; particulate concentration of 7.3 mg/m^3). No preneoplastic or neoplastic lesions developed in the respiratory tracts of the animals. Combining diesel exhaust with other contaminants from uranium ore mines failed to produce cocarcinogenic effects.

In conclusion, the earlier studies on gasoline and diesel engine exhausts clearly indicate that the organic particulate extracts of engine exhausts contain materials with carcinogenic activity. They also showed that in spite of the presence of these carcinogens, the inhalation of whole engine exhaust by laboratory animals did not induce neoplastic lesions in the respiratory tract. This is most likely due to the very low concentration of carcinogenic agents in diluted exhaust aerosols from gasoline and diesel engines. Other factors to consider are the low doses of exhaust reaching the relevant target cells in the lungs of the animals, and the resistance (or effective physiological defense) of the respiratory tract tissues to the carcinogens contained in engine exhausts.

CURRENT STUDIES ON DIESEL ENGINE EXHAUST MATERIALS

Carcinogenic Agents in Diesel Engine Exhausts--Chemical Studies

Diesel exhaust is largely a mixture of incomplete combustion products of diesel fuel, lubricating oils, and the degradative products of lubricating oils. The exhaust is composed of a gas phase and a particulate phase. Little is known about the presence of carcinogenic agents in the gas phase. Early studies by Kotin and coworkers (1954a, 1963) indicated that certain volatile unsaturated hydrocarbons may give rise to epoxides and peroxides in the atmosphere. Some of the former have been shown to be carcinogenic in experimental animals (see review by Lawley, 1976). The volatile organics that may be present in diesel exhaust might also form peroxides in the atmosphere.

Nitrogen oxides (NO_x) are another gas-phase component that may play a role in the formation of carcinogens in urban atmospheres. The incremental addition of NO_x from diesel exhaust to the ambient air is one of the causative agents of urban air pollution. Oxides of nitrogen can serve as precursors of carcinogenic N-nitrosamines by reacting either in the atmosphere (Pitts et al., 1978a) or in vivo with secondary and tertiary amines (Magee et al., 1976). Several of the nitrosamines that may be so formed have been found to be potent carcinogens in laboratory animals (Magee et al., 1976). Studies by Fine (1978) have shown, however, that urban air is only a minor source of man's exposure to N-nitrosamines. Pitts and coworkers (1978b) recently demonstrated in laboratory studies that polycyclic aromatic hydrocarbons may react with NO_x to form nitro-polycyclic aromatic hydrocarbons, some of which are highly mutagenic.

Additional trace components found in the gas phase of diesel exhaust are formaldehyde and other volatile aldehydes (e.g., acrolein and acetaldehyde) (Mentser and Sharkey, 1977). Several of these substances are known to inhibit lung clearance (Battista, 1976). In the case of formaldehyde, a recent inhalation study has shown that chronic exposure of rats for up to 18 months to 15 ppm of this compound induces a high incidence of nasal carcinomas (36/84 rats). In concentrations of 2 and 6 ppm it did not induce nasal tumors (Swenberg et al., 1980).

Studies by Sawicki and coworkers (1960) have shown that urban air contains volatile alkylating agents. Presently, it is uncertain whether and to what degree the volatile components of diesel exhaust will contribute to the alkylating potential of urban air pollutants. However, recently β-propiolactone, a carcinogenic alkylating agent (Lawley, 1976), has been reported in diesel exhaust (Menster and Sharkey, 1977). Analytical data concerning the chemical nature of many of the other minor constituents are unavailable at present. Based on current knowledge, however, it is not unlikely that diesel exhaust also contributes to other volatile urban pollutants with biological activity (e.g., certain nitroparaffins, nitro-olefins, and *o*-methylarylamines) (Deichmann *et al.*, 1965).

Considerably more is known about the chemical nature of the organic extracts of diesel exhaust particulates. One group of chemicals that has been studied in some detail are the polycyclic aromatic hydrocarbons. As early as 1955, Kotin and coworkers reported the presence of benzo[*a*]pyrene, benzo(*ghi*)perylene, and anthanthrene in diesel exhaust. They described an increase in the emission of polycyclic aromatic hydrocarbons with increasing engine speed, engine load, and inefficiency of engine operation. Lyons (1962) and Choudhury and Bush (1979) identified a large number of polycyclic aromatic hydrocarbons in diesel exhaust using spectrophotometric methods, and Hanson and coworkers (1979) reported extensive identification studies on polycyclic aromatic hydrocarbons from diesel engine exhaust. These authors also reported that *S*- and *N*-heterocyclic aromatic hydrocarbons were present in the polycyclic aromatic hydrocarbon concentrates from diesel exhaust particulate extracts.

Data on the emission of polycyclic aromatic hydrocarbons from the exhaust of diesel engines operated on the European driving schedule have been reported by Grimmer and Bohnke (1978). Among the compounds identified were known carcinogens for mouse skin and for the subcutaneous tissues of both mice and rats. These include benz[*a*]anthracene, chrysene, cyclopenteno [*c*,*d*]pyrene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, and three dibenzopyrenes. Several investigators have reported the quantitative determination of polycyclic aromatic hydrocarbons, particularly of benzo[*a*]pyrene, in diesel exhaust. Huisingh and coworkers (1979) compared the concentrations of benzo[a]pyrene in the exhaust particulate extracts from four different diesel engines with that from a gasoline engine equipped with a catalytic converter. The benzo[a]pyrene concentrations found in cigarette-smoke condensate, coke-oven effluent, and roofing tar were included in this comparison. (See Table 3.1.)

In a comparison of the engines listed in Table 3.1, by far the highest concentration of benzo[a]pyrene (ng per mg of particulate) was found in the exhausts from the Nissan diesel and the Mustang II gasoline engines. The latter was equipped with a catalytic converter and was operated on unleaded gasoline at a richer-than-normal stoichiometry in order to produce a sufficient volume of sample.

It is noteworthy that in this study the benzo[a]pyrene concentrations in the exhaust particulates from the Nissan and Mustang II engines were one or two orders of magnitude greater than those from the Volkswagen Rabbit, the Oldsmobile, and the Caterpillar engines. If, based on the benzo[a]pyrene concentration data, the amount of the substance emitted per mile traveled is calculated using the particulate emission rates for each engine (see Table 3.2), then the engines can be ranked in the following order (µg benzo[a]pyrene per mile based on the test conditions of this study): Nissan engine 31.7 µg; Volkswagen Rabbit, 0.8 µg; Oldsmobile, 0.2 µg; and Mustang II, 0.2 µg.

Williams and Swarin (1979) compared the average benzo[a]pyrene emission rate per mile (as representative of total carcinogenic polycyclic aromatic hydrocarbons) of seven gasoline engines without catalytic converters, four with them, and two light-duty diesel engines. The average rates were: diesel engines, 2.7 µg; gasoline engines without catalytic converters, 2.7 µg; and gasoline engines with catalytic converters, 0.07 µg. (See Figure 3.1.) A comparison of the General Motors Research Laboratories and the EPA studies with respect to benzo[a]pyrene emissions is shown in Table 3.3.

The various diesel exhaust particulate organic fractions were tested for their mutagenicity in several laboratories (Choudhury and Doudney, 1979; Huisingh *et al.*, 1979; Lofroth, 1979; Siak *et al.*, 1979). (See page 22 *et seq.*) Some of the active fractions were further analyzed and in certain cases their chemical constituents were identified. The weakly polar neutral fraction was the most active in the Ames mutagenicity assay with the

TABLE 3.1 A Comparison of the Benzo[*a*]pyrene Present in Exhaust Particulate Extracts and in Other Sources

Sample Source	Extractable Matter (percent)	Benzo[*a*]pyrene Benzo[*a*]pyrene, ng / extract, mg	 Benzo[*a*]pyrene, ng / particulate, mg
		Diesel Engine Exhaust	
Caterpillar	26-27	2	0.5
Nissan	4-8	1,170	96.2
Oldsmobile	12-17	2	0.4
Volkswagen Rabbit	18	26	4.6
		Gasoline Engine Exhaust	
Mustang II	39-43	103	44.1
		Comparative Sources	
Cigarette-smoke condensate	--	--	<1.0
Coke-oven emissions	5-10	478	31.5
Roofing tar	>99	889	889.0

Source: Huisingh *et al.* (1979).

TABLE 3.2 Particulate Emission Rates for Five Vehicle Engines

	Emission Rate	
Sample Source	grams/horsepower/hour	grams/mile
	Diesel Engine Exhaust	
Caterpillar	0.72	--
Nissan	--	0.33
Oldsmobile	--	0.52
Volkswagen Rabbit	--	0.18
	Gasoline Engine Exhaust	
Mustang II	--	0.0053

Source: Huisingh *et al.* (1979).

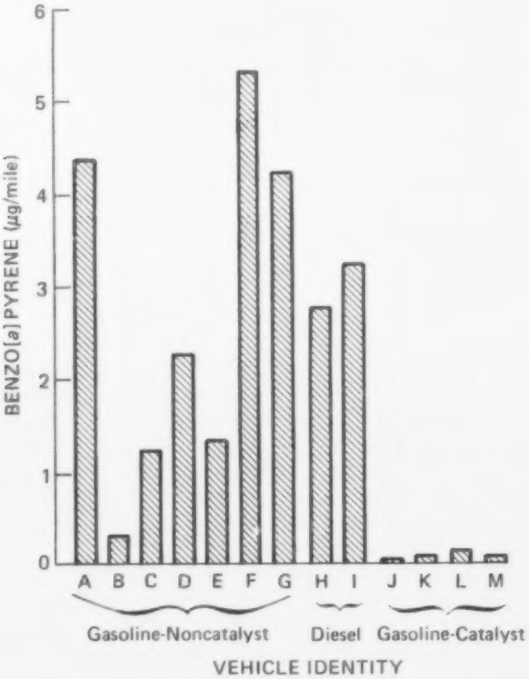

FIGURE 3.1 Benzo[*a*]pyrene emission rates determined under test conditions proposed for light-duty diesel vehicles. (Source: Williams and Swarin, 1979.)

TABLE 3.3 Average Benzo[*a*]pyrene Emissions (μg/mile) from Four Automobile Engines Found by Two Sets of Studies

General Motors Research Laboratories Study		EPA Studies	
Diesel (Oldsmobile):	2.7	Nissan diesel:	31.7
		Volkswagen diesel:	0.8
		Oldsmobile diesel:	0.2
Gasoline without catalytic converter:	2.7		
Gasoline with catalytic converter:	0.07	Mustang II gasoline with catalytic converter:	0.2

Sources: General Motors Research Laboratories studies by Williams and Swarin (1979). EPA Studies by Huisingh *et al*. (1979).

TA98 and TA100 *Salmonella* strains. Thus far, oxygenated polycyclic aromatic hydrocarbons, including phenols, quinones, dicarboxylic acid anhydrides, and nitropolycyclic aromatic hydrocarbons, have been identified. The compounds 3-nitrofluoranthene and 1-nitropyrene have been detected and appear to be highly mutagenic. Thus, carcinogenicity data on these two compounds would be most desirable (Lofroth, 1979).

Other investigators have shown that the nonpolar neutral subfraction of the exhaust particulates of a two-stroke diesel engine contains C_{10}-C_{24} paraffin hydrocarbons, and those of a four-stroke diesel engine, C_{24}-C_{40} paraffins (Rodriguez *et al*., 1979). Several of the lower molecular weight hydrocarbons can act as cocarcinogens on mouse skin when applied in high concentrations together with benzo[*a*]pyrene (Bingham and Falk, 1969; Van Duuren and Goldschmidt, 1976).

The particulate phase of the exhaust of heavy-duty diesel engines is reported to contain between 500 and 10,000 ppm of nickel. Nickel carbonyl, which is volatile, as well as several nonvolatile nickel compounds, has been shown to be carcinogenic in experimental animals. In addition, workmen exposed to the inhalation of nickel compounds have been found to be at increased risk from cancer of the lung and nasal cavity (International Agency for Research on Cancer, 1976b; National Research Council, 1975).

In summary, chemical studies have shown that the gas as well as the particulate phase of diesel exhaust contains carcinogenic chemicals (or their precursors). Gas-phase constituents of gasoline and diesel engine exhaust have not been sufficiently studied. The particulate matter contains carcinogenic polycyclic aromatic hydrocarbons. Benzo[a]pyrene determinations in the exhausts of various gasoline and diesel engines (benzo[a]pyrene as representative of carcinogenic polycyclic aromatic hydrocarbons) lead to the following tentative conclusions: Benzo[a]pyrene emissions (μg/mile) from gasoline engines without catalytic converters can vary by more than one order of magnitude depending on engine type alone; Catalytic converters effectively reduce the amount of benzo[a]pyrene in the emissions from gasoline engines; Benzo[a]pyrene emissions from diesel engines can vary by two orders of magnitude depending on engine design alone; and Benzo[a]pyrene emissions (μg/mile) from the diesel engines having the lowest benzo[a]pyrene emissions are the same order of magnitude as those from gasoline engines with catalytic converters.

Thus, these data suggest that through engine design modifications alone (not including modifications in fuel composition, in engine operation, and in after-treatments), the amount of benzo[a]pyrene emitted per mile can be reduced to approach the level emitted per mile in the exhaust of gasoline engines equipped with catalytic converters.

To estimate the tumorigenic potential of diesel engine exhaust, chemical-analytical studies are needed on the identification and quantitative determination of carcinogenic, tumor-initiating, and cocarcinogenic agents in both the gas and the particulate phases.

Biological Studies with Extracts from Engine Exhaust Particulates

In Vitro Studies with Mammalian Cell Transformation Systems

The systems that are being used for in vitro transformation with diesel exhaust particulate extracts involve the morphological transformation of Balb/c 3T3 mouse cells (see page 31 et seq.) and the enhancement of viral transformation in Syrian hamster embryo cells. The latter assay is relatively new (Casto et al., 1979). Because the polycyclic aromatic hydrocarbons (and possibly other substances in the extracts), which are important components of diesel exhaust materials, need metabolic activation by microsomal enzymes, such tests are usually conducted with and without the addition of (rat) liver microsomes.

The most common endpoint scored in the in vitro assays is "morphological transformation" because it is the first nonbiochemical indication that a heritable change has taken place in the cells. This type of change is, however, not identical with "neoplastic transformation" (i.e., tumor formation upon inoculation of these cells into suitable hosts), which is a later-occurring event. The quantitative and temporal relationships between various markers of transformation and the development of tumorigenicity have been described and discussed in detail by Barrett and Ts'o (1978) for the Syrian hamster embryo cell system.

There are certain limitations to the mammalian transformation assay. Particularly where complex toxic test mixtures such as engine exhaust materials are being investigated, these limitations involve reproducibility, quantitation, establishment of dose-response relationships, etc. Nevertheless, mammalian cell transformation assay systems are useful as indicators of the carcinogenicity of the test substances, as screening assays, and as systems providing supportive information for other bioassays. A substance that repeatedly scores positive in one or more transformation assays is highly suspect of being carcinogenic in vivo.

Even though the transformation assay with Balb/c 3T3 cells carried out by Curren and coworkers (1979) showed no clear dose-response relationships for a variety of test substances, transforming activities with and without the addition of microsomal activation were clearly demonstrated for several engine exhaust materials. Extracts from the Nissan diesel engine and the Mustang II gasoline engine as well as from roofing tar and coke-oven effluents were positive. The responses to these four types of materials all appeared to be of the same order of magnitude. No activity above background was demonstrated for exhaust extracts from Oldsmobile and Caterpillar diesel engines.

Studies on the enhancement of the viral transformation of Syrian hamster embryo cells by automobile exhaust extracts and other materials were carried out by Casto and coworkers (1979). Their assays showed fairly good dose-response effects for all materials tested. Judging by the lowest concentration of test material affecting the enhancement of viral transformation, all of the samples tested showed some activity, although the activities of the Caterpillar and Oldsmobile exhaust extracts were borderline. (See Table 3.4.) The Nissan, Mustang II, and Volkswagen Rabbit exhaust extracts appeared to be within the same range, with the Nissan ranking the highest. Cigarette-smoke condensate and particularly coke-oven effluents and roofing tar (materials that were used for comparison purposes) were clearly more active, with roofing tar ranking highest.

Thus, in principle, studies with the two transformation assays show the same trends: namely, that the Nissan diesel engine and the Mustang II gasoline engine (with catalytic converter, but run inefficiently) have borderline transformation activity. The other tested diesel engine exhaust fractions have definite transforming activity, and roofing tar and coke-oven effluent have strong transforming activity.

In Vivo Studies with the Mouse Skin Carcinogenesis Assay

The mouse skin carcinogenesis assay is one of the most widely used experimental models for mechanistic studies of carcinogenesis as well as for bioassay studies to determine the carcinogenic activity of various test substances. Its principles as well as its utility have been described in numerous articles, including a recent paper by Slaga and coworkers (1979). This assay makes it possible to distinguish various types of carcinogenic

TABLE 3.4 The Potency of Organic Extracts of Diesel Exhaust and Related Environmental Emissions for the Enhancement of Viral Transformation in Hamster Embryo Cultures

		Enhancement[b]		
Sample	LECT[a]	LECT	HECT	Ranking[c]
Roofing tar	3.1	1.63	2.58	1
Coke-oven emissions	7.8	1.69	4.04	2
Cigarette-smoke condensate	31.2	1.63	5.60	3
Nissan	62.5	1.91	4.07	4
Mustang II	125.0	1.90	3.06	5
Volkswagen Rabbit	125.0	1.75	2.40	6
Oldsmobile	250.0	1.48[d]	1.51	7
Caterpillar	500.0	1.35	1.35	8
	Controls			
Benzo[*a*]pyrene	0.12	1.70	8.97	N.A.
N-methyl-*N*'-nitro-N-nitrosoguanidine	0.25	4.10	4.60	N.A.

[a] Lowest effective concentration tested (µg/ml) that induced significant enhancement ($p < 0.01$) of adenovirus transformation.

[b] Enhancement was determined by dividing the transformation frequency of extract-treated cultures by that obtained from solvent-treated cultures of Syrian hamster embryo cells. HECT = maximal enhancement obtained at the highest effective concentration tested in these experiments.

[c] Ranking was based on the lowest concentration causing significant enhancement. Where two samples were positive at the same concentration, the one inducing more enhancement was ranked higher.

[d] Indicates values positive at the 5 percent level of significance, but not at the 1 percent level.

Source: Casto *et al.* (1979).

activities, such as initiating* and promoting† activities, complete carcinogenic activity, and cocarcinogenic‡ activity.

The scope of the studies in mouse skin carcinogenesis projected by Slaga and coworkers (1979) is summarized in Table 3.5. At present, data from the collaborative study between the EPA and the Oak Ridge National Laboratory are only available from the tumor-initiation studies. As Slaga and coworkers showed with two classic carcinogenic polycyclic aromatic hydrocarbons (7,12-dimethylbenz[a]anthracene and benzo[a]pyrene), response data for tumor-initiating activity can be obtained within 15 weeks using Sencar mice, by scoring skin papillomas. The papilloma incidence per mouse is a fairly good predictor of the carcinoma incidence at one year (see Table 3.6). For some polycyclic aromatic hydrocarbons, their relative tumorinitiating ability is comparable to their complete carcinogenic activity (see Table 3.7). The data available on the skin tumor-initiating activities of exhaust extracts from several diesel engines as well as from five other sources are summarized in Table 3.8. Five dose levels of each materials were tested. The maximum amount was 10 mg.

At the time of this report, data were available only on the tumor-initiating activities of the extracts of the particulate matter of coke-oven effluents and of a Nissan diesel engine, after 14 weeks of promotion.

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* An initiator is an agent that causes a permanent change in exposed tissue resulting in tumor formation when (and only when) the tissue is subsequently exposed (for long periods of time) to a promoting agent. Many carcinogens act as initiators at subcarcinogenic doses.

† A promoter is a noncarcinogenic agent that, when repeatedly applied following the exposure of tissue to an initiator, will bring about tumor formation in that tissue. (Promoters are active only when applied after initiation.) The processes of initiation and promotion are most typically described in the two-stage mouse skin carcinogenesis model.

‡ A cocarcinogen is any noncarcinogenic agent that will enhance the tumor response induced by a carcinogen (sometimes used to refer to those agents that will do so only when applied simultaneously with the carcinogen.)

TABLE 3.5 Objectives of the Diesel Research Program

Determine:	Complete carcinogenesis
	Cocarcinogenesis
	Tumor initiation
	Tumor promotion
Samples:	Oldsmobile diesel engine exhaust extracts
	Nissan diesel engine exhaust extracts
	Volkswagen Rabbit diesel engine exhaust extracts
	Caterpillar diesel engine exhaust extracts
	Mustang II gasoline engine exhaust extracts (with catalytic converter)
	Coke-oven emissions
	Roofing tar
	Cigarette-smoke condensate
Protocol:	SENCAR mice
	40 males and 40 females
	5 dose levels
Standards:	Benzo[*a*]pyrene for complete carcinogenesis and tumor initiation
	Pyrene for cocarcinogenesis with benzo[*a*]pyrene
	Tetradecanoyl phorbol acetate for tumor promotion

Source: Slaga *et al*. (1979).

Benzo[*a*]pyrene was used as the positive control. Data were not available for the tumor-initiating activities of the other five remaining materials being tested.

The biostatistical evaluation of the tumor yields is given in Table 3.8. The interpretation of the experimental results reported in this table is difficult because the experiments are, as yet, incomplete. As in most of the other bioassay studies, this study also compares the combustion materials on a weight-to-weight basis without considering the actual environmental concentrations.

As in the *in vitro* transformation studies, the exhaust materials from the Nissan engine showed the highest activity of all the engine exhaust materials when compared with roofing tar and coke-oven emissions, and the extract from the Caterpillar engine the lowest. In the two-stage tumor-induction study, the initiating activity of benzo[*a*]pyrene is two or three orders of

TABLE 3.6 Dose-response Studies of the Ability of 7,12-Dimethylbenz[a]anthracene (DMBA) and Benzo[a]pyrene (BaP) to Initiate Skin Tumors in SENCAR Mice

Initiator	Dose[a] (nmoles)	Number of Papillomas per Mouse at 15 Weeks	Percent of Mice with Papillomas at 15 Weeks	Percent of Mice with Carcinomas at 50 Weeks
DMBA	100	22.0	100	100
DMBA	10	6.8	100	40
DMBA	1	3.2	93	22
DMBA	0.1	0.5	20	5
BaP	200	7.5	100	55
BaP	100	3.2	78	30
BaP	50	1.4	60	18

[a] The mice were treated one week after initiation with twice-weekly applications of 5 μg of tetradecanoyl phorbol acetate.

Source: Slaga *et al*. (1979).

magnitude greater than that of the various emissions. A shortcoming of the study by Slaga and coworkers (1979) is that the polycyclic aromatic hydrocarbon concentrate from the engine exhaust emissions is compared with the total particulate matter of cigarette smoke (condensate) and not with the polycyclic aromatic hydrocarbon concentrate from cigarette smoke.

An extensive series of studies on the carcinogenicity of gasoline engine exhaust condensate (without catalytic converter) and diesel engine exhaust condensate was carried out in the Federal Republic of Germany by the Working Group for the Investigation of the Carcinogenic Burden by Air Pollution in Man (Brune *et al*., 1978; Grimmer and Bohnke, 1978; Misfeld, 1979; Misfeld and Timm, 1978). These studies determined the complete carcinogenicity of a mixture of 15 polycyclic aromatic hydrocarbons, of gasoline engine exhaust condensate, of diesel engine exhaust condensate, and of benzo[a]pyrene. The test materials were applied epicutaneously twice weekly for the lifespan of the mice. The amount of diesel exhaust condensate applied each time was calculated to contain a quantity of tumorigenic polycyclic aromatic hydrocarbon comparable to that applied with the gasoline exhaust condensate. (See Table 3.9.)

TABLE 3.7 Comparison of Complete Carcinogenesis and Tumor Initiation in Mouse Skin[a]

Compound	Relative Potency[b] Complete Carcinogenesis (carcinomas)	Relative Potency[b] Tumor Initiation (papillomas)
7,12-Dimethylbenz[*a*]-anthracene (DMBA)	100	100
3-Methylcholanthrene	50	50
Benzo[*a*]pyrene (BaP)	30	30
2-Hydroxybenzo[*a*]pyrene	30	30
7-Bromomethyl-12-methyl-benz[*a*]anthracene	20	20
Benzo[*a*]pyrene-7,8-oxide	20	20
Dibenz[*a*,*h*]anthracene	20	20
Benzanthracene	5 ± 5	5
Dibenz[*a*,*c*]anthracene	0	3
Pyrene	0	0
Benzo[*a*]pyrene-4,5-oxide	0	0
Anthracene	0	0

[a] This is a summary of over 100 compounds which shows that an excellent qualitative and quantitative correlation exists between complete carcinogenesis and tumor initiation in mouse skin.

[b] Relative potency was determined from dose-response data. DMBA was given a maximum value of 100.

Source: Slaga *et al.* (1979).

The skin tumor yields are presented in Table 3.10. As shown in Figure 3.2, the survival rates of the mice in the groups varied significantly with the materials applied. Therefore, the author transformed the tumor data into age-standardized rates (Misfeld, 1979). (See Table 3.11 and Figure 3.3.)

Finally, the relative carcinogenic potencies for diesel exhaust condensate, gasoline exhaust condensate, the 15 polycyclic aromatic hydrocarbons, and benzo[*a*]pyrene were compared. (See Table 3.12.) The author concluded that on a weight basis, and in his experimental setting, the exhaust condensate of a gasoline engine without a catalytic converter is about 42 times more tumorigenic on mouse skin than that of a diesel engine.

TABLE 3.8 Comparative Skin Tumor-Initiating Activities of Extracts of Diesel Exhaust and of Related Environmental Emissions[a]

Sample	Papillomas/mouse/mg[b] (14 weeks)	R^2
Caterpillar diesel engine	0	--
Nissan diesel engine	0.258	0.996
Oldsmobile diesel engine	0.115	0.95
Mustang II gasoline engine	0.09	--
Cigarette-smoke condensate	0	--
Roofing tar	0.182	0.999
Coke-oven emissions	0.307	0.876
Benzo[a]pyrene	46.2	0.984

[a] Forty males and 40 females were initiated with the various samples and promoted one week later by twice-weekly applications of 2 μg tetradecanoyl phorbol acetate.

[b] The values represent the slope from the linear regression analysis of the dose-response studies and measure of fit (R^2).

Source: Slaga et al. (1979).

Biological Studies with the Whole Particulate Fraction from Engine Exhaust

In Vitro Studies with Human Fibroblasts

Normal human fibroblasts and fibroblasts from xeroderma pigmentosum patients were used as the test system to assess the cytotoxicity of diesel engine exhaust particulates. Xeroderma pigmentosum fibroblasts are deficient in repairing damaged DNA; normal human fibroblasts are not. A comparison of the reduction in cloning efficiency of normal human fibroblasts with that of xeroderma pigmentosum cells following their incubation with the test material gives an indication of the DNA damage. Using this system, McCormick and coworkers (1979a, 1979b) showed that both the unextracted particles from an Oldsmobile 350 diesel engine and their organic extracts (using methylene chloride) reduce the cloning efficiency of xeroderma pigmentosum cells (50 percent survival with 100 μg/ml and 48 mg/ml equivalent, respectively), while the extracted

TABLE 3.9 Experimental Design Testing the Carcinogenic Activity of Automobile Exhaust Condensate, of Diesel Exhaust Condensate, and of Subfractions

Test Group Substance	Applied Individual Dose (mg)	Animals in Test Group
Control without treatment	--	80
Control solvent	--	80
Benzo[*a*]pyrene	0.00385	65
	0.00769	65
	0.01538	65
Gasoline engine exhaust condensate	0.29	80
	0.88	80
	2.63	80
Diesel engine exhaust condensate	4.30	80
	8.60	80
	17.15	80
15 polycyclic aromatic hydrocarbons of gasoline engine exhaust condensate[a]	0.0035	80
	0.0105	80

[a] Fifteen polycyclic aromatic hydrocarbons of gasoline engine exhaust condensate (weight proportion as in condensate): benzo[*a*]phenanthrene (0.08 μg), cyclopentenopyrene (1.85 μg), benz[*a*]anthracene (0.09 μg), chrysene (0.21 μg), benzo[*b*]fluoranthene (0.17 μg), benzo[*k*]-fluoranthene (0.06 μg), benzo[*j*]fluoranthene (0.09 μg), benzo[*a*]pyrene (0.30 μg), 1,12-methylene-benzo[*e*]pyrene (0.14 μg), 10,11-methylenbenzo[*a*]pyrene (0.05 μg), dibenz[*a*,*j*]anthracene (0.10 μg), indeno[1,2,3-*cd*]pyrene (0.21 μg), dibenz[*a*,*h*]-anthracene (0.02 μg), M 300A (0.07 μg), M 300B (0.06 μg).

Source: Misfeld (1979).

particulates showed no reduction in cloning efficiency. Thus, the diesel exhaust particulate suspensions (unextracted) showed similar cytotoxicity to that of the organic diesel exhaust particulate extracts. (See pages 36 and 39.)

These studies indicate that the materials adsorbed on diesel exhaust particulates that can be extracted by organic solvents are bioavailable. The elution of these materials from the particulates probably occurs intracellularly. Several studies have been conducted to

TABLE 3.10 Mouse Skin Bioassay for Carcinogenic Activity of the Organic-Phase Condensates from Gasoline Engine Exhaust and Diesel Engine Exhaust: Tumor Yields

Test Group Substance	Applied Individual Dose (mg)	Evaluable Animals in Test Group	Average Length of Survival During Test (weeks)	Animals with Tumors		Tumor Induction Period (weeks)
				Absolute	Percent	
Control without treatment	--	78	85	0	0.0	0
Control solvent	--	78	76	0	0.0	0
Benzo[_a_]pyrene	0.00385	64	78	21	0.0	74
	0.00769	64	68	39	60.9	61
	0.01538	64	55	57	89.1	44
Gasoline engine exhaust condensate	0.29	78	80	8	10.3	72
	0.88	79	78	35	44.3	72
	2.63	78	64	65	83.3	52
Diesel engine exhaust condensate	4.30	75	80	0	0.0	0
	8.60	76	81	2	2.6	102
	17.15	71	77	9	12.7	76
15 polycyclic aromatic hydrocarbons of gasoline engine exhaust condensate	0.0035	77	78	1	1.3	91
	0.0105	75	76	29	38.7	73

Source: Misfeld (1979).

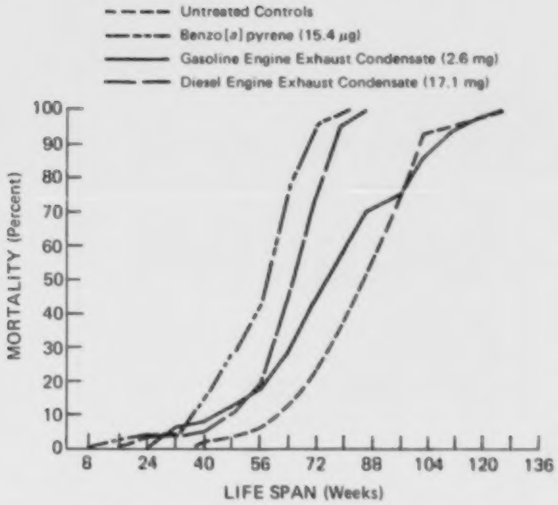

FIGURE 3.2 Survival rates for some of the mouse test groups in studies on the carcinogenicity of automobile exhaust condensates. (Source: Misfeld, 1979.)

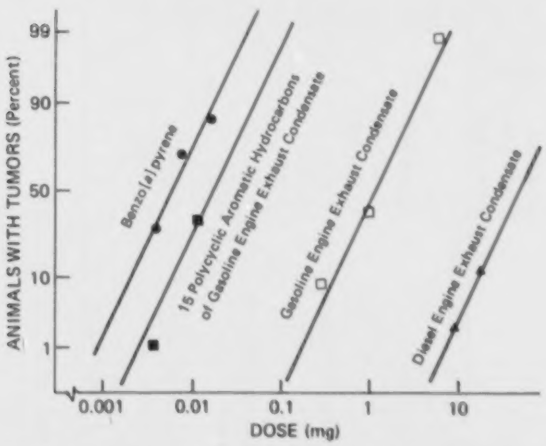

FIGURE 3.3 Mouse skin bioassay for carcinogenic activity of the organic-phase condensates from gasoline and diesel engine exhausts: age-standardized test rates. (Source: Misfeld, 1979.)

TABLE 3.11 Mouse Skin Bioassay for Carcinogenic Activity of the Organic-Phase Condensates from Gasoline Engine Exhaust and Diesel Engine Exhaust: Age-standardized Test Rates

Test Group Substance	Applied Individual Dose (mg)	Evaluable Animals in Test Groups	Animals with Tumors	
			Raw Frequencies	Standardized Frequencies
Control without treatment	--	78	0.0	0.0
Control solvent	--	78	0.0	0.0
Benzo[a]pyrene	0.00385	64	32.8	28.4
	0.00769	64	60.9	70.2
	0.01538	64	89.1	85.8
Gasoline engine exhaust condensate	0.29	78	10.3	8.5
	0.88	79	44.3	34.8
	2.63	78	83.3	93.5
Diesel engine exhaust condensate	4.30	75	0.0	0.0
	8.60	76	2.6	1.9
	17.15	71	12.7	11.5
15 polycyclic aromatic hydrocarbons of gasoline engine exhaust condensate	0.0035	77	1.3	1.1
	0.0105	75	38.7	33.0

Source: Misfeld (1979).

TABLE 3.12 Relative Carcinogenic Potencies of Diesel Engine Exhaust Condensate, a Mixture of 15 Polycyclic Aromatic Hydrocarbons, and Benzo[a]pyrene Related to Gasoline Engine Exhaust Condensate, and of Benzo[a]pyrene Related to Diesel Engine Exhaust Condensate

Substance	Relative Potency	95 Percent Confidence Limits
Gasoline engine exhaust condensate	1.0	--
15 polycyclic aromatic hydrocarbons of gasoline engine exhaust condensate	68.0	46.0-101.0
Benzo[a]pyrene	187.0	127.0-286.0
Diesel engine exhaust condensate	0.024	0.015-0.035
Diesel engine exhaust condensate	1.0	--
Benzo[a]pyrene	7,950.0	5,210.0-13,300.0

Source: Misfeld (1979).

evaluate the release of biologically active materials from diesel exhaust particulates in the presence of physiological fluids. In one such study (Siak *et al.*, 1979), incubation of diesel exhaust particulates in body fluids did not result in their extractions. More recent studies by King and coworkers (1980) indicated, however, that "...substantial mutagenic activity is released from diesel particulates upon incubation with serum and lung cytosol."

In Vivo Studies with the Pulmonary Adenoma Assay

The pulmonary adenoma assay is one of the more sensitive, quantitative, reproducible *in vivo* tumorigenesis assays. It responds to a wide spectrum of carcinogens and has, therefore, broad application (Shimkin and Stoner, 1975). It seems particularly well-suited for the study of the unextracted particulates from engine exhaust (and similar materials), because the intraperitoneally injected particulates are retained in the peritoneal cavity for extended periods of time. This increases the chance that materials on the particulates will be eluted from them and enter the systemic circulation. However, as discussed earlier (see page 60), caution must be used when interpreting the data obtained with the pulmonary adenoma assay because the mechanism underlying the enhancement of pulmonary adenoma development by carcinogens is not understood. The assay, nevertheless, is an important screening test.

A study has been proposed (U.S. EPA, 1979b) to determine the relative carcinogenicity of diesel exhaust particulates using the pulmonary adenoma assay on Strain A mice. The materials are to be injected intraperitoneally into mice shortly after weaning. Except for the 2 control chemicals, urethan (1 x 10 mg) and benzo[_a_]pyrene (1 x 25 mg), the test substances are to be injected 3 times per week, for a total of 24 injections of 1 mg of material per injection (55 mice per group). The test "mixtures" are coke-oven effluent, roofing tar, cigarette-smoke condensate, and diesel exhaust particulates. The animals are to be sacrificed at 9 months of age to determine the number of pulmonary adenomas.

This experiment is designed after the Andervont and Shimkin (1940) model (see also Shimkin and Stoner, 1975). It should provide important information about the carcinogenicity of diesel exhaust particulates, and it should have relevance to the question of bioavailability regarding materials adsorbed on the particulates.

In Vivo Studies with Intratracheal Instillation Assays

Intratracheal instillation of test materials into experimental animals is a valuable experimental procedure, particularly when it is essential to determine the carcinogenicity of the substances for respiratory tract tissues. The method does have serious limitations, however, particularly when the test materials are toxic and cause pneumonia, bronchopneumonia, and even abscess formation.

The distribution of intratracheally injected materials within the various sizes of airways is uncontrolled. Although the tumor response following intratracheal injection of carcinogens such as benzo[_a_]pyrene is roughly dose-related (Feron _et al_., 1973; Saffiotti _et al_., 1972), close comparisons of the carcinogenicity of materials differing in physicochemical properties and tissue toxicity are problematic, due to differences in tissue distribution, in local dose, in retention time, in degree of tissue destruction, etc. A typical example of the effects of the physical characteristics of intratracheally injected materials is the low tumorigenic activity of small (<1μ) ferrous oxide carrier particulates laden with benzo[*a*]pyrene, and the high tumorigenic activity of large benzo[*a*]-pyrene-laden particulates (5 to 10μ) (Henry *et al.*, 1974). In this case, the carcinogenic efficacy of the two test preparations is primarily determined by particulate size.

The intratracheal instillation method seems to be most appropriate to determine the carcinogenicity of engine exhaust substances for respiratory target tissues (as opposed to other target tissues). This method might also yield some information concerning comparisons of different exhaust and effluent materials.

In order to evaluate the carcinogenic activity of diesel exhaust particulates in the respiratory tract of animals, Shefner and coworkers (1979) initiated an intratracheal instillation experiment in Syrian golden hamsters. For the intratracheal application of diesel exhaust particulates and their extracts, the methodology developed was one using two test materials (Graf, 1979).

For the first material, diesel exhaust particulates from an Oldsmobile 350 test engine were collected on glass fiber filters. The resulting flake-like sheets of aggregated particulates were powdered to sizes amenable for the assay, and were suspended in saline containing 0.5 percent gelatin, 7 percent propylene glycol (as the wetting agent), and ferrous oxide (Fe_2O_3) as the carrier dust.

The second material was prepared by using the dichloromethane extract of the diesel exhaust particulates in an emulsion with ferrous oxide as the carrier dust and propylene glycol and sorbitan as wetting agents. The extract alone and a ferrous oxide suspension in saline served as negative control materials. Benzo[*a*]-pyrene with ferrous oxide served as a positive control material. The intratracheal instillation was achieved according to the method of Saffiotti and coworkers (1972). In the preliminary experiment, which served as a guide for a large-scale lifetime study, 15 weekly instillations of 12 test materials were made. The 15 x 1 mg extracts of the Oldsmobile diesel exhaust particulates contained

30 ng of benzo[a]pyrene. Five weeks after the last application the animals were sacrificed. (See Table 3.13.)

The following results were reported:

- The diesel exhaust particulates and their extracts plus ferrous oxide induced only minor or no signs of systemic toxicity;
- There was no significant nonrespiratory pathology;
- Lesions in the lung were common in the treated hamsters;
- Adenomatous hyperplasia were more severe and extensive in the extract-treated animals than in the controls;
- More adenomas were observed in the extract-treated groups than in the controls; and
- Metaplasia were observed in some of the animals injected with the extract, as well as severe multifocal reactive pneumonitis with hyperplasia and some evidence of cellular atypia.

A long-term tracheal instillation study with Syrian golden hamsters is planned by the same laboratory and will be based on the data from the short-term test.

Biological Studies with Whole Engine Exhaust
(Inhalation Studies)

The importance of the inhalation studies with whole engine exhaust lies in the fact that they are the only experiments testing the mixture of gases, aerosols, and particulates as it is being generated by the test engines. Also, these studies are the only ones in which animals are exposed in a physiological manner. However, as discussed on page 60, inhalation exposure tests are not sensitive for assaying complex mixtures (Laskin and Sellakumar, 1974).

An inhalation study was carried out with Strong Lab Strain A (A/Strong) and Jackson Lab Strain A (A/Jackson) mice (Orthoefer et al., 1979). The animals were exposed to exhaust from a Nissan diesel engine for 20 hours per day, 7 days per week for 7 to 8 weeks (particulate concentration was 6.3 to 6.9 mg/m^3). The animals were sacrificed after 26 to 30 weeks. The results are summarized in Table 3.14. In comparing the groups exposed to nonirradiated exhaust with the control group

TABLE 3.13 Experimental Design of Intratracheal Instillation into Syrian Golden Hamsters[a]

Group	Treatment[b]	Dose[c] per Instillation (mg)	Frequency (per week)	Remarks
1	DEPs[a]	5	2	--
2	DEPs	5	1	--
3	DEPs	3	1	--
4	DEPs	1	1	--
5	DEPs + Fe_2O_3	5	2	1 death
6	DEPs + Fe_2O_3	5	1	--
7	DEPs + Fe_2O_3	3	1	1 death
8	DEPs + Fe_2O_3	1	1	--
9	Benzo[a]pyrene + Fe_2O_3	3	1	19 deaths
10	Benzo[a]pyrene + Fe_2O_3	1.5	1	5 deaths
11	Fe_2O_3 in vehicle	5	1	3 deaths
12	Vehicle control	—	1	5 deaths
13	Shelf controls	(no instillations)		

[a] Each hamster given 1 instillation per week for 15 weeks, followed by observation for 5 weeks. Each group contained 50 hamsters.

[b] DEPs = diesel exhaust particulates.

[c] Dose refers to quantity of DEPs, benzo[a]pyrene, or of Fe_2O_3 when only single materials were administered. When test materials were given with Fe_2O_3, the concentration is that of each component administered as 1:1 mixtures by weight.

Source: Shefner *et al*. (1979).

exposed to clean air, a slight, but statistically insignificant, difference existed in the average number of pulmonary adenomas per A/Strong mouse. This trend was not observed in the A/Jackson mice exposed to the same exhaust.

In a second inhalation test in the same study by Orthoefer and coworkers (1979), 8 groups of A/Strong male mice, 100 mice in each group, were used. The starting age of the mice was 3 to 6 weeks. Six of the groups were exposed to clean air or diesel exhaust 20 hours daily, 7 days per week, for 33 weeks, and 2 of the groups were exposed for 38 weeks. For this experiment, the diesel exhaust was diluted with filtered air in a ratio of 1:13 and passed through dynamic flow irradiation chambers (to simulate sunlight). The resulting diesel exhaust contained particulates at a concentration of 6.87 mg/m^3. This study showed "...no significant differences between the treated groups and the control groups." No tumors other than pulmonary tumors were seen.

In a third inhalation test in the same study by Orthoefer (1979), groups of 60 female A/Strong mice were exposed to diesel exhaust generated by a 6-cylinder 196-$in.^3$ Nissan engine using number 2 diesel fuel, or to clean air for 20 hours per day, 5 days per week for 7-1/2 months. The concentrations of volatile compounds and particulate matter (6.4 mg/m^3) in the exposure chamber were monitored. In addition, 2 other groups of the same sex and strain of mice received a 1-mg dose of urethan (positive control) intraperitoneally prior to their exposure to the diesel exhaust or to the clean air. At the end of the exposure period, the number of adenomas in the lungs was determined. A random number of lung surface nodules and any questionable lesions were processed for histopathological evaluation in support of the macroscopic lung nodule counts.

The survival of the 4 groups varied from 84 to 95 percent. Four animals in the group exposed only to air and 14 exposed only to diesel exhaust had lung adenomas. The group that received urethan and was subsequently exposed to air had 9 lung adenomas, while the group that received urethan and was then exposed to diesel exhaust had 22 tumors. Thus, diesel exhaust or the combination of urethan and diesel exhaust enhance the lung adenoma incidence in female A/Strong mice.

Based on these studies, exposure to diesel exhaust enhanced the pulmonary adenoma incidence in mice that

TABLE 3.14 Lung Tumors in Mice Exposed to Diesel Engine Exhaust (Nissan), in Mice Treated with Urethan, and in Controls

Treatment	Dose		Survivors/ Initial Number	Mice with Lung Tumors	Average Number of Tumors/Mouse
		Strong Lab Strain A[a]			
Nonirradiated exhaust	6.3 mg/m^3		19/25	7/19	0.63
Irradiated exhaust	6.9 mg/m^3		22/25	6/22	0.27
Control	--		22/25	3/22	0.13
Urethan[b]	20 mg		23/25	23/23	Multiple
		Jackson Lab Strain A[c]			
Nonirradiated exhaust	6.3 mg/m^3	Males	16/20	5/16	0.31
		Females	18/20	6/18	0.50
Control	--	Males	18/20	5/18	0.33
		Females	18/20	11/18	0.66
Urethan[b]	20 mg		17/20	17/17	Multiple

[a] For Strong Lab Strain A, x^2 (for average number of tumors per mouse) = 2.96 with 2 df p = 0.28.

[b] Urethan used as positive control.

[c] For Jackson Lab Strain A, x^2 (for average number of tumors per mouse) = 5.31 with 3 df p = 0.150.

Source: Orthoefer _et_ _al_. (1979).

had either not received a prior injection of urethan or those that had received a 1-mg injection. Because of the low numbers of tumors involved, the statistical significance of this result is, however, borderline.

An inhalation study has been initiated at the EPA's Center Hill Laboratory (U.S. EPA, 1979a). The goal of this study is to determine if the exposure to diluted diesel exhaust induces preneoplastic changes and tumors in the upper respiratory tract and lungs of Syrian golden hamsters. Two groups of 100 female Syrian golden hamsters each were exposed to diesel exhaust diluted with clean air and to clean air alone, respectively. (No experimental details are yet available.) The study was completed in October 1980.

Inhalation studies proposed to test the carcinogenicity of diesel exhaust in organs other than the lung, particularly in the liver, involve many variables. The results of the experiments proposed by Shinozuka and Lombardi at the University of Pittsburgh, to use rats that have been partially hepatectomized and placed on a choline-deficient diet, will not be easily interpretable.

Other proposed studies would expose Strain A mice and their F_1 generation to diesel exhaust, and examine tumor incidence as well as reproductive parameters (U.S. EPA, 1979f). Fetal tissues as well as tissues of newborn mice are highly susceptible to carcinogenic agents. Therefore, these proposed studies seem conceptually sound. The exposure of breeding animals and their offspring may possibly represent a highly sensitive bioassay system. It is recommended, however, that mouse strains other than the A strain, such as the C3H mouse, also be considered for inclusion in the study. The A strain mouse is selected primarily for its lung tumor response. However, under the conditions of the proposed experiments, other organs might also be potential targets for diesel exhaust carcinogens. The C3H mouse, for example, is highly susceptible to liver tumor induction.

Chronic inhalation studies are being conducted by Heinrich and coworkers (1979), using Syrian golden hamsters as experimental animals. The animals are exposed to exhaust from a Daimler-Benz engine for 8 hours per day, 5 days per week. The exhaust is diluted with clean air in ratios of 1:3, 1:5, or 1:10 (4 to 17 mg/m^3 particulate mass concentration). A preliminary 5-month study has been completed in which half of the hamsters were exposed to whole exhaust and half to the exhaust minus particulates. A lifetime exposure study with 18 groups of 48 animals each is now under way. The variables in this study are clean air, total exhaust, and exhaust without particulates. The animals are also receiving injection of diethylnitrosamine (1.5 or 4.5 mg/kg, once, subcutaneously), intratracheal instillation of dibenz[*a,h*]anthracene (20 x 0.3 mg or 20 x 0.1 mg), or intratracheal instillation of pyrene (20 x 0.1 mg). The hamsters pretreated with diethylnitrosamine, dibenz[*a,h*]anthracene, or pyrene are subsequently exposed to diluted control exhaust or, for control purposes, to air only. The purpose of the injection of the two carcinogens (diethylnitrosamine or dibenz[*a,h*]-anthracene with the pyrene serving as a noncarcinogen control) is to produce a low respiratory tract tumor incidence that might either permit detection of a promotion effect of the diesel exhaust or that might increase the chance of detecting a low tumorigenic activity of the inhaled exhaust. No tumor data are available from these investigations. A shortcoming of this study is that there are too many groups each containing a relatively small number of animals.

Another chronic inhalation study with diesel engine exhaust is currently being carried out in a cooperative study by General Motors Research Laboratories and Wayne State University (Puro, 1980). Fischer 344 rats and Hartley guinea pigs were exposed to air-diluted diesel exhaust generated by a 5.7-liter Oldsmobile engine. The exhaust concentrations tested contain 250, 750, and 1,500 $\mu g/m^3$ of particulates. After various exposure times, the lungs of the exposed animals were examined for changes by light microscopy. Animals in groups of 3 to 7 were exposed (individually caged) for 20 hours per day, 5-1/2 days per week for periods of 3, 6, or 12 months or lifetimes. Pigmented macrophages were found in the lungs of both the rats and the guinea pigs at every concentration and after 3, 6, and 12 months of exposure. After 6 months at the 750 $\mu g/m^3$ exposure level some early fibrotic changes were seen in regions with macrophage clusters. Type 2 cell reactions were seen in alveoli containing collections of pigmented alveolar macrophages. After 12 months of exposure, there was no indication of a neoplastic response, as determined by light microscopic examination of the bronchial and bronchiolar epithelium.

Charboneau and McCauley (1979) studied the ability of lung microsomes from exposed animals to oxidize benzo[*a*]pyrene to polar metabolites, and the ability of such metabolites to bind to DNA. The purpose of this study was to see if the lung microsomes of diesel exhaust-exposed animals would be activated by this exposure. Such enhanced activity could conceivably increase the likelihood of metabolic activation or deactivation of environmental carcinogens. Fischer 344 rats were exposed to diesel exhaust with particulate levels of 250μ g/m^3 and 1,500μ g/m^3, generated by a 5.7-liter Oldsmobile diesel engine. At the high diesel exhaust exposure level, the lung weights were increased after exposure for 1 year. (See page 108.)

An important finding in the Charboneau and McCauley study was that in animals exposed for 1 year the ability of microsomes to produce polar metabolites of benzo[*a*]pyrene was reduced. At present, the significance of this finding in terms of a greater or lesser cancer risk cannot be assessed because of the complexity of the benzo[*a*]pyrene activation and detoxification pathways. These findings were supported by studies carried out by Cantrell and coworkers (1979), who found that A/Jackson mice exposed to diesel exhaust showed a reduction in benzo[*a*]pyrene metabolism in their lungs.

A large-scale inhalation study has been initiated with Strain A/Jackson mice, Fischer 344 rats, and randomly bred Syrian golden hamsters by the Southwest Foundation for Research and Education and the Southwest Research Institute, San Antonio, Texas, under contract with General Motors Research Laboratories. In a short-term (3-month) study, more than 2,000 animals of the 3 species are exposed either to diluted diesel exhaust (1,500μ g/m^3 particulates) or to filtered air, for 20 hours per day, 7 days per week. Recovery rates are being ascertained for 6 months following the exposure period. Subsequently, a large-scale, 15-month study will be initiated with mice, rats, and hamsters. The animals will be held in inhalation chambers aerated with filtered air and with diluted diesel exhaust containing 250, 750, and 1,500μ g/m^3 particulates, respectively.

These inhalation experiments are based on past experiences by General Motors Research Laboratories (Barnhart *et al.*, 1979). The plan to expose the animals daily for 20 hours to diluted diesel exhaust is commendable. The investigators should consider the possibility of increasing the concentration of diesel exhaust particulates because studies by Orthoefer and coworkers (1979) have shown that Strain A mice will tolerate up to 6,400 $\mu g/m^3$ particulates when exposed 20 hours per day, 5 days per week. Inhalation experiments by Heinrich and coworkers (1979) indicated that Syrian golden hamsters will accept up to approximately 7,000 to 8,000 $\mu g/m^3$ (7 to 8 hours per day, 5 days per week). Finally, it is recommended that the long-term exposure should not be limited to 15 months but should be terminated only when the animals are near the end of their lifespan (Interagency Regulatory Liaison Group, 1979).

Two inhalation experiments with exhaust from internal combustion engines have been initiated or are planned by Stoeber and coworkers (1980) at the Fraunhofer Institute, University of Muenster, West Germany. In the first study, an estimated 3,000 mice, rats, and hamsters will be exposed to three dilutions of exhaust from a Volkswagen (or Audi) diesel engine operated on the European driving cycle. Major emphasis will be placed on the exposure of hamsters with or without pretreatment with intratracheally administered benzo[a]pyrene or with a nontumorigenic four-ring aromatic hydrocarbon. The goal is to evaluate the potential of diesel exhaust as a complete respiratory carcinogen, and also as a tumor promoter. Ongoing short-term inhalation experiments will provide the details needed for the design of the 2-year study, such as particulate concentration, daily exposure time, dose of benzo[a]pyrene as tumor initiator, etc.

The second study, initiated by the West German automobile industry and the West German EPA, is currently in the planning stage. Rats and/or hamsters (1,800) will be exposed to diluted gasoline engine exhaust. The dilution rate will be determined by the highest tolerated carbon monoxide level. Some of the animals will be pretreated by intratracheal instillation of a carcinogenic or a noncarcinogenic polycyclic aromatic hydrocarbon or by injecting the hamsters with a subthreshold level of N-nitrosodiethylamine. The results of this study will be of importance for a comparison with the data from diesel exhaust inhalations at the same institute.

It is hoped that these 2 inhalation studies will be extended from 5 days weekly (in the ongoing long-term study) to 7 days weekly, and that major emphasis will be on the hamster inhalation study. Several possible shortcomings were noted in the design of these studies. Three hamsters are housed in a single cage, making it possible for the animals to hide their nostrils in each others fur, thus reducing the deposition of particulates in the lungs.

In the case of the second study, with approximately 10 to 15 groups, the major goal of the project, namely the determination of a "no effect" level, may not be realized because the number of animals per group does not seem to be large enough. The pretreatment of hamsters with *N*-nitrosodiethylamine will induce tracheal papillomas in animals exposed to any irritating chemical inhalant, irrespective of its tumorigenic potential (Fern, 1979; Hoffmann *et al*., 1979).

A large-scale inhalation experiment is planned with Fischer rats and Syrian golden hamsters by the Battelle Institute, Geneva, Switzerland, under contract with the Comité Des Constructeurs d'Automobiles du Marché Commun (1980). The animals will be exposed to exhausts from Volkswagen and Renault gasoline and diesel engines. The proposed lowest dilution of diesel exhaust (1:15) is expected to deliver 4 to 8 mg/m^3 of particulates. At present, only a few details of the program are tentatively set. It is hoped that the planned weekly exposure regimen will be increased from 5 to 7 days. The intratracheal instillation of benzo[*a*]pyrene as a tumor initiator for assaying the tumor-promoting potential of combustion materials will result in meaningful data, provided that, in the proposed negative control, pretreatment with the cocarcinogen pyrene is replaced by pretreatment with a truly nontumorigenic polycyclic aromatic hydrocarbon.

SUMMARY AND CONCLUSIONS

In summarizing the research findings of the current experimental studies related to the potential carcinogenicity of diesel engine exhausts, it must be emphasized that much of the recent work is still incomplete. Thus, final conclusions cannot be drawn as yet. In fact, some of the most important *in vivo* carcinogenesis studies are currently in progress. Nevertheless, based on the available data, some definitive and some tentative conclusions can be drawn.

- Extracts from diesel (and from gasoline) engine exhaust particulates contain carcinogenic materials. This is supported by many older as well as current chemical and biological studies. The carcinogenic activities of these extracts appear to be two or three orders of magnitude lower on a weight-to-weight basis than that of benzo[*a*]pyrene, a representative carcinogenic polycyclic aromatic hydrocarbon.

- Whether whole engine exhaust particulates (from gasoline and/or diesel engines) are carcinogenic is as yet unknown. Existing data are limited and are either negative or ambiguous. Important studies are under way, involving intraperitoneal injection into Strain A mice and intratracheal injection into hamsters.
- Neither diesel nor whole gasoline engine exhaust has so far been found to be carcinogenic when inhaled by laboratory animals. This negative finding is based mostly on previous studies with a variety of animal species (mice, rats, hamsters, and dogs). Chronic large-scale inhalation studies that are presently under way have not, as yet, yielded information concerning carcinogenicity.
- Variable fuel composition and engine operating characteristics may, according to *in vitro* cell transformation assays, turn out to be significant determining factors in the biological activity of diesel engine exhaust materials. It is, at the moment, uncertain whether the *in vivo* carcinogenesis assays will show the same trends.
- Based on the skin carcinogenesis studies of Misfeld and Timm (1978), Brune and coworkers (1978), and Misfeld (1979), in which the carcinogenic activity of diesel and gasoline engine exhaust extracts (the gasoline engine used was not equipped with a catalytic converter) were compared, it appears that per mile traveled (or on weight-to-weight basis) the amount of carcinogenic material emitted might be within the same order of magnitude for both engine types.
- Based on the data presently available from EPA-supported skin tumor-initiation studies (Slaga *et al.*, 1979), the activities of extracts of roofing tar, of coke-oven effluent, and of the exhaust materials from one gasoline engine and two diesel engines are all within the same order of magnitude (per unit weight of material tested). It should be remembered, however, that this comparison does not take into consideration the environmental concentrations of the various effluents to which man is actually exposed.
- Mouse skin carcinogenicity data and other quantitative bioassay data can be used to estimate the

relative carcinogenicity of organic extracts of both diesel exhaust and related environmental emissions. These estimates can then be combined with available epidemiological data on the related environmental emissions in attempting to assess the potential human cancer risk from exposure to diesel engine emissions. Harris (1981) has performed such an assessment. It is based upon epidemiological data on occupational exposure to coke-oven and roofing-tar emissions, along with the results of initiation promotion experiments on mouse skin and oncogenic transformation experiments from ongoing EPA studies. The resulting estimates of the potential range of lung cancer risk are of the same order of magnitude as those obtained from an epidemiological study of lung cancer among diesel bus garage workers. (See page 126 *et seq*.) It must be recognized that this method of comparative risk assessment assumes that the relative potencies of environmental emissions are preserved across human and nonhuman biological systems. This assumption, however, is based on many unknowns; the practical value of risk assessments relying on such assumptions is limited in view of interspecies and interorgan differences in factors such as bioavailability, particulate distribution, extractability and clearance of active organics, target site of action, metabolism, and genetic repair mechanisms.

Both past and current studies either leave a number of important questions unanswered or provide insufficient information:

- Essentially no information is available concerning the carcinogenicity of gas-phase components. Attempts should be made to learn more about these substances.
- Further identification of the components of diesel exhaust fractions that contain mutagenic and carcinogenic activity is needed. The chemical characterization of these materials would be useful to guide future attempts at engine modification to reduce carcinogenic emissions.
- One of the major questions that has not been adequately resolved concerns the *in vivo* bioavailability of the toxic substances adsorbed on diesel exhaust particulates. In general, available information seems to indicate that the organic substances are tightly bound to the carbonaceous core. Although they are extractable with polar solvents such as methylene

chloride, the evidence from several experiments suggests that these materials are not bioavailable. However, *in vitro* studies, including one with *xeroderma pigmentosum* cells, as well as two inhalation studies, suggest that some of the materials associated with diesel exhaust particulates are bioavailable. Studies should be designed to measure, for example, the elution of polycyclic aromatic hydrocarbons from the diesel exhaust particulates *in vivo*.

- Future investigations need to place more emphasis on comparative studies of the relative carcinogenicity of light-duty diesel engine exhaust and the exhausts of gasoline engines (with and without catalytic converters). This is essential because the decisive question is whether diesel engines add more potentially carcinogenic agents to the environment per mile driven than gasoline engines under the same load.
- At present, the most quantitative carcinogenesis data can be expected to be derived from the skin carcinogenesis and intraperitoneal injection studies with Strain A mice. Future studies should also make use of another highly sensitive bioassay model, i.e., the newborn mouse (Asahina *et al.*, 1972).

To make specific recommendations based on the mostly incomplete data seems unwarranted. However, three facts seem to emerge from the various chemical and biological studies in spite of all the shortcomings of the assay systems used: diesel exhaust contains traces of carcinogenic materials; the carcinogenic activity of the materials in diesel exhaust appears to be low; and engine design appears to greatly affect the carcinogenic activity of diesel exhaust particulates.

4 PULMONARY AND SYSTEMIC EFFECTS

Diesel exhaust contains both a number of recognized volatile toxic substances (see Introduction) and a high concentration of carbonaceous particles. Various hydrocarbons, some of them known carcinogens, are adsorbed on the surfaces of these particles.

Assessing the potential pulmonary and systemic effects of exposure to diesel exhaust must be done by emphasizing the anticipated morbidity rather than mortality. In this chapter, the panel examines the past, ongoing, and proposed research on the potential pulmonary and systemic problems related to the various components of diesel exhaust, both gaseous and particulate. The following are the major areas of concern:

- The size of the diesel exhaust particulates (<1 µm) is small enough to be readily deposited deep in the lungs. The particulates thus have the potential for carrying toxic substances into the lungs where they may be leached off and transported via the systemic circulation into other organs;
- The extent to which the various materials are soluble in body fluids--that is, their bioavailability and its relationship to their ultimate systemic toxicity;
- The extent to which the gaseous portion of the exhaust could adversely alter the ambient levels of certain pollutants (CO, SO_x, NO_x, O_3, and volatile aldehydes), depending on the nature of the fuel and the condition of the engine; in addition, the extent to which components of the gaseous portion would undergo atmospheric chemical reactions (as in photochemical smog) that create other toxic volatile substances (e.g., peroxyacetyl nitrate);

- The effects of diesel exhaust on specific populations such as those with cardiopulmonary diseases, asthmatics, the very young, and the old; and the effects of differences in levels of exposure to both the gaseous and the particulate components under varying conditions of physical activity such as recreational exercise and hard labor;
- The noxious effects of the total exhaust, especially odor and visibility, and possibly eye irritation, on various exposed populations;
- The potential for an increase of cardiovascular diseases resulting from the possible incremental addition of CO to present ambient levels;
- The potential for a rise in infectious diseases in the very young (<2 years) by the additional NO_2 in the ambient air; and
- The suggested evidence of adverse behavioral effects caused by the influence of certain of the gaseous components on the human central nervous system.

METHODOLOGIES USED TO STUDY PULMONARY AND SYSTEMIC EFFECTS

Information on lung damage, systemic effects, and other possible toxic effects of diesel exhaust have been obtained with laboratory animals. In 1977, the EPA issued a Precautionary Notice recommending that "... precautionary measures be taken to minimize human exposure to concentrated diesel exhaust or its collected products." This notice has been interpreted by some as meaning that research involving exposure of human subjects to diesel exhaust should not be conducted because of the potential carcinogenic hazard. Because the effect of this notice has been to prohibit such research, further clarification is needed. If epidemiological studies are to be of value, controlled clinical studies must be considered. Man has been and will continue to be exposed to certain levels of diesel exhaust, and controlled studies at comparable levels need to be performed. Animal studies are useful for at least two major purposes:

- They may inform as to the toxic potential of diesel exhaust; and
- They provide guidance to judge the conditions of exposure at which adverse human health effects may occur.

Routes of Exposure

The most meaningful data on pulmonary and systemic health effects of diesel exhaust are likely to be obtained from inhalation studies. The physical and chemical properties of diesel exhaust may vary widely depending on engine operating conditions (Kotin et al., 1955). Variables include engine type (displacement, brake horsepower), fuel quality (e.g., sulfur content), cycling of operation modes, and load (selection of test mph and cold or hot engine start). The output of particulates, NO_x, CO, and polycyclic aromatic hydrocarbons is strongly influenced by engine wear, combustion efficiency, type of fuel, and fuel:air ratios. The presence or absence of gas exhaust recirculation, turbocharging, or particulate traps further affect diesel exhaust characteristics both qualitatively and quantitatively.

The preferred method of exposing animals to exhaust is a dynamically operated whole-body exposure chamber (Beethe et al., 1979; U.S. DHEW, 1978; WHO, 1978a). The characteristics of exhaust and chamber concentrations can be evaluated with accuracy (Soderholm, 1979; Williams and Begeman, 1979; Williams and Chock, 1979). Animals exposed by inhalation will provide information about toxic potential, possible dose-effect relationships, and possible time-effect relationships.

Other routes of exposure would appear to be of lesser value in assessing the pulmonary or systemic toxicity of diesel exhaust emissions. Intratracheal instillation allows the delivery of a precisely known amount of the test sample into the respiratory tract. It may, however, lead to uneven and focal distribution with unrealistic concentrations of topical materials (Brain et al., 1976). The cutaneous application of the condensates of diesel exhaust particulates might yield some information on the overall toxicity, provided that enough of the material adsorbed to the particulates can be absorbed through the skin. Administration by gavage may be used to investigate whether diesel exhaust components are likely to be absorbed into the systemic circuit from the gastrointestinal tract. This would aid in the assessment of the potential for adverse health effects from material cleared from the lungs via the mucociliary escalator, and subsequently swallowed.

Experimental Design

Selecting the most appropriate animal species for inhalation studies includes consideration of comparative respiratory tract morphology, the presence or absence of lung disease, and the similarity of physiological responses to those in man (WHO, 1978b).

A potential problem relates to the possibility that the gaseous products of diesel engine operations (i.e., NO_x, CO, SO_x, and other air pollutants) may actually be increased over present ambient levels. If this occurs, pulmonary function may be compromised beyond the levels presently accepted for air pollutants. This necessitates designing experiments that provide information on the toxic potential and the no-effect level of diesel exhaust.

To study the toxic potential, various animals should be exposed to high levels of test material, and the most suitable animal model (e.g., rodents, dogs, subhuman primates, donkeys) must be chosen. If the probability of a given exposure producing a toxic effect in man is 1 percent, then 300 animals must be exposed in order to produce the same lesions in at least 1 test animal with 95 percent confidence. If the probability of producing toxic effects in man is 0.1 percent, then 3,000 animals must be tested (Zbinden, 1973). Establishing no-effect levels may require a different approach. Studies designed to ascertain no-effect levels, which are ultimately used to establish safety, will require tests with large numbers of animals exposed to several dose levels. Accordingly, studies with small laboratory animals, such as mice, rats, and hamsters, are the only practical means of carrying out such safety tests.

Inhalation studies with diesel exhaust emissions, lasting a few hours, may be used to assess acute biological effects of engines operated under different running conditions (Pattle et al., 1957). Studies from 1 to 3 months in duration are useful for their flexibility in design. They make it possible to obtain a maximum amount of information about toxic potential, and provide the opportunity to study the development and reversibility of pulmonary and other lesions (WHO, 1978b). Such short-range tests could also be useful to evaluate acute exposures in individuals of various ages and the relation of diesel exhaust to the occurrence of preexisting cardiopulmonary diseases. Chronic or lifetime studies to examine chronic pulmonary and systemic effects should be incorporated into those studies designed primarily to evaluate carcinogenicity. Guidelines for these carcinogenicity studies in rodents have been presented in a report of the National Cancer Institute (National Cancer Institute, 1976).

In selecting endpoints for a toxicity study, the decision must be made whether information is wanted primarily on mechanisms or on dose-response time relationships. Studies to elucidate mechanisms often require using the most advanced and complex techniques. Valuable information can be obtained by using comparatively few animals under conditions of exposure where an unequivocal toxic response can be anticipated. By contrast, dose-response time studies require larger numbers of animals.

It is practical to choose comparatively simple, although strictly quantitative, endpoints. Possible endpoints for assessing lung damage by morphological (Dungworth et al., 1976), physiological (Wilson et al., 1976), and biochemical (Mustafa and Tierney, 1978; Witschi and Cote, 1977) methods have recently been critically reviewed. Furthermore, observations of weight gain, food and water consumption, and signs of acute or chronic illness will provide information about system and organ damage. Upon termination of the experiment, extensive histopathology (Zbinden, 1973) will provide additional data.

AVAILABLE INFORMATION ON THE PULMONARY AND SYSTEMIC HEALTH EFFECTS OF DIESEL EXHAUST IN ANIMALS

The data base on animals exposed to the major individual gaseous components of diesel exhaust has been reviewed and summarized in several documents (National Research Council, 1977a, 1977b, 1978). Less information is available on animals exposed to diesel exhaust under controlled conditions, and practically no information exists that would allow a comparison to be made among several studies run under identical conditions. The preliminary data available with regard to the potential health hazards of diesel exhaust are summarized in the succeeding sections. However, it should be realized that of all the documents reviewed on the pulmonary and systemic effects of diesel exhaust, only four have been published in refereed journals (Abraham et al., 1980; Lee et al., 1980; Pattle et al., 1957; Yamazaki, 1969).

All other information in this area provided to the Health Effects Panel has consisted of preliminary and unrefereed reports. The panel notes that all of these need to be subjected to confirmation and peer review.

Under contract from the U.S. Department of Energy, additional research is under way at the Lovelace Biomedical and Environmental Research Institute (Inhalation Toxicology Research Institute) in eight areas. These are: lung function and structure, tracheal mucous clearance, connective tissue metabolism, immunological response, airway and tissue biochemistry, DNA and lipid synthesis, aryl hydrocarbon hydroxylase and cyctochrome P_{450} induction, and lung bacterial infection. A final research program is being developed.

General Toxicity

The acute toxicity of undiluted exhaust produced by a diesel engine was studied in mice, guinea pigs, and rabbits exposed by inhalation for 5 hours (Pattle *et al.*, 1957). When the engine was operated under light engine load, with air intake restricted, all the animals died from acute CO poisoning (1,700 ppm) during the exposure period. When the engine was run with a heavy engine load (410 ppm CO), approximately 50 percent of the mice died during the exposure; half the rabbits and guinea pigs survived the actual exposure period but died within the next 7 days.

Acute and delayed mortality were reduced greatly when the engine was run under moderate engine load but with a worn fuel injector. If the engine was in optimum working condition and run under light load, no acute mortality was seen. An attempt was made to attribute acute lethality to particular components of diesel exhaust. The researchers concluded that under light-load conditions, aldehydic irritants (e.g., acrolein) and, to a lesser extent, NO_x, were the main toxic agents. Under a moderate load, NO_x was the chief toxic component, and, under a light load with restricted air intake, CO and, to a lesser extent, aldehydic irritants were the main toxic components. Therefore, acute toxicity of diesel exhaust appeared to be associated with the gaseous components. In those studies, particulate concentrations were one to two orders of magnitude higher than in all subsequent studies.

In most subsequent studies, animals were exposed to diluted diesel exhaust at much lower concentrations and for much longer times. In the experiments designed at General Motors Research Laboratories, the chamber concentrations of particulates expressed as diesel engine exhaust were 250, 750, and 1,500 μg/m^3 (Schreck et al., 1979). In studies done at the EPA Health Effects Research Laboratory in Cincinnati, the average chamber concentration of particulates was approximately 6,000 μg/m^3 (Orthoefer et al., 1979). Other relevant studies (Heinrich et al., 1979; U.S. DHEW, 1978) were conducted under similar conditions. Chamber concentrations of CO were at or below 50 ppm and of NO_x were between 10 and 80 ppm. Under these conditions, animals usually survived quite well for prolonged periods of time, even if exposed for up to 22 hours per day, 5 to 6 days per week. In a study with rats exposed for 45 or 54 days, weight gain was comparable to controls (Moore et al., 1978). No excessive mortality was found in Strain A mice exposed to particulate concentrations of from 6,000 to 7,000 μg/m^3 for 7 weeks (Orthoefer et al., 1979).

Hamsters exposed for up to 600 days to an average chamber concentration of 7,000 μg/m^3 of particulates showed survival curves similar to controls (U.S. DHEW, 1978). In a different study, hamsters tolerated particulate concentrations of up to 17,000 μg/m^3 for prolonged periods of time (Heinrich et al., 1979). Cats exposed to 6,000 μg/m^3 for 8 hours per day for 1 year survived through the entire period (U.S. EPA, 1979b).

The few routine toxicological evaluations done in these experiments (e.g., body weight gain, hematology) failed to reveal any gross untoward effects of the diesel exhaust. Exposure of small laboratory animals to dilutions of 1:7 or less of diesel exhaust is thus compatible with long-term survival and is without any apparent gross toxicity.

The administration of diesel exhaust by other routes of exposure also had no obvious toxic effects. In hamsters, repeated intratracheal instillation of exhaust particulates (5 mg, once or twice per week) did not affect weight gain, except transitorily in the high-dose group, and it did not produce more than anticipated mortality (Shefner et al., 1979). Intraperitoneal injection of 705 μg of particulates per week into mice, a dose slightly less than would be inhaled within a week in an atmosphere of particulates at a concentration of 6,000 μg/m^3, produced no evidence of toxicity as indicated by general appearance and growth rate (Orthoefer _et al_., 1979).

Pulmonary Effects

Pathology

The pathological changes in the lungs of small animals (mice, guinea pigs, and rabbits), following acute exposure for 5 hours to toxic concentrations of diesel exhaust, are pulmonary congestion, hemorrhage, and edema. In those animals surviving for several days, the changes observed were the ones commonly seen in acute chemical pneumonitis, such as consolidation with alveolar collapse and occasional emphysematous changes (Pattle _et al_., 1957).

Morphological changes in animals exposed to diesel exhaust for prolonged periods of time have been reported in a few studies. A thorough analysis of respiratory tract lesions was done on a group of 105 male Syrian golden hamsters exposed for 6 hours per day, 5 days per week, for up to 14 months or longer (U.S. DHEW, 1978). Chamber concentrations were: NO_2, 4 to 6 ppm; SO_2 and aliphatic aldehydes, less than minimum detectable (1 ppm); carbon monoxide, 50 ppm; and particulates, 7.3 mg/m^3. In the animals that survived the longest, the most conspicuous macroscopic and microscopic findings were extensive carbon retentions in the lungs. In those cases, the alveoli were filled with black material.

Morphological signs of pulmonary emphysema were found with comparatively high frequency in the hamsters after 2 to 4 months of exposure, together with acute interstitial pneumonitis, alveolar septal hyperplasia, and moderately severe bronchiolization of the alveolar epithelium. The early lesions did not increase greatly in severity between 4 and 11 months, although their incidence remained high. Lung damage from chronic exposure to diesel exhaust is thus comparatively nonspecific. The accumulation and retention of black material and damage to the epithelial cells of the alveoli were the most conspicuous pathological findings. The most striking feature in the lungs of these animals was a severe accumulation of particulate-laden macrophages (U.S. DHEW, 1978).

The same observation was made in rats and guinea pigs exposed up to 12 months to diesel exhaust particulate concentrations of 250, 750, or 1,500 $\mu g/m^3$ for 20 hours per day, 6 days per week (Barnhart _et al._, 1979; Puro, 1980). The pulmonary response appeared to follow a standard pattern: inhaled particulates were first engulfed by alveolar macrophages and then became sequestered within phagolysosomes.

In the lungs of animals exposed to diesel exhaust, the number of alveolar macrophages increased more than twofold and covered a larger surface of the alveoli than in normal lungs. Diesel exhaust particulates were found in free alveolar macrophages, in interstitial phagocytic cells, and even in alveolar Type I epithelial cells. Where macrophages congregated, there was often a proliferation and increase in the number of Type II alveolar epithelial cells. Large clusters of particulate-bearing macrophages eventually accumulated around the ends of terminal bronchioles, and some collagenous fibers appeared in the alveolar septa. Particulates were also seen within lymphatic vessels, where they lay inside or outside of phagocytotic cells and eventually accumulated within regional lymph nodes (Puro, 1980).

A large variation in the sizes of macrophages in the lungs of guinea pigs exposed to diesel exhaust was observed (Barnhart _et al._, 1979). In control animals, the average macrophage diameter was 13 μm (range 9 to 14 μm), whereas in the group exposed to the highest dose, macrophages had an average diameter of 25 μm (range 10 to 44 μm). However, studies with cells harvested from the lungs of exposed guinea pigs revealed that the ingested particulate material was not found to be cytotoxic and remained confined within membranous structures (Chen _et al._, 1980). Macrophages, even if filled with particulates, remained as viable (trypan blue exclusion test) as those from control lungs. They were still capable of ingesting latex particles but at a slower rate than control macrophages. After a 12-month exposure to 250 μg of diesel exhaust, the capability of pulmonary macrophages to ingest foreign particles was reduced by 20 to 30 percent. Intracellular acid phosphatase was also decreased (Weller _et al._, 1980).

The pulmonary defense system of exposed mice was challenged with infectious agents (Campbell _et al._, 1979). Animals were exposed for from 2 hours to several weeks to 7,000 $\mu g/m^3$ of diesel exhaust. Upon challenge with an aerosol of _Streptococcus pyogenes_, more animals died in the exposed group than in the controls;

increased post-infection mortality was seen only after 2 to 6 hours of exposure to diesel exhaust, and appeared to become higher in animals exposed for either 2 or 46 weeks.

Diesel exhaust that was irradiated photochemically increased the susceptibility to infection. In animals challenged with a viral pathogen (A/PR8-34) or given *Salmonella typhimurium* by gavage, diesel exhaust exposure did not enhance infectivity. The data did provide some evidence that pulmonary defense mechanisms might be compromised following exposure to diesel exhaust. Whether increased susceptibility to bacterial infection was due to overload of the macrophage system with diesel exhaust particulates or was instead caused by the NO_x present in the exhaust remains to be established.

Deposition and Clearance

Respirable particles deposited in the airways and in the deep regions of the lung are removed by various clearing mechanisms (e.g., the mucociliary escalator and absorption) (Abraham *et al.*, 1980; Brain *et al.*, 1976). Although information on deposition and clearance is of prime importance in estimating the total body burden of inhaled agents, knowledge of the fate of inhaled diesel exhaust aerosols is still limited.

Data available so far indicate that the initial deposition efficiency of freshly generated diesel exhaust particulate is 15 ± 6 percent of the inhaled dose (Vostal *et al.*, 1979). This is about half the amount that would be predicted by applying a generally accepted model of particulate deposition and clearance (International Commission on Radiological Protection, 1966). Expected lung doses of inhaled diesel exhaust particulates in rats exposed for 1 year (20 hours per day, 5-1/2 days per week) to 250, 750, or 1,500 $\mu g/m^3$ were calculated to be 5,000, 32,000, and 60,000 μg, respectively. The fate of inhaled and deposited particulates was further studied following long-term exposure (Vostal *et al.*, 1979).

Black material in the lungs accumulated and persisted in animals exposed to both low and high doses of diesel exhaust (250 and 1,500 $\mu g/m^3$). In both groups, it appeared that normal clearance mechanisms became exhausted after about 3 to 4 months of exposure. From then on, material accumulated and persisted in the lungs, with the higher dose leading to a much larger lung burden of inhaled materials. Particulate-laden macrophages moved towards the mucociliary escalator. Macrophages laden with carbonaceous material or even free particulate aggregates were also found near the peribronchiolar and periarterial lymph aggregates and later in the lymphatic vessels and large lymph nodes of the hilar region.

Eventually, the lymph nodes became filled with particulate-engorged macrophages. Cellular structures disintegrated and large aggregates of particulate matter were dispersed throughout the medullary cords with increasing accumulation toward the hilus. This indicates that diesel exhaust particulates deposited in the deep regions of the lungs are removed from the lungs by the mucociliary escalator and the lymphatic system. The particulates, or materials adsorbed to them, may thus reach the general circulation via these routes.

Pulmonary Function

Several studies have been initiated to examine whether or not acute or chronic exposure to diesel exhaust may change pulmonary function in laboratory animals. In one study, diesel exhaust particulate material was collected and aerosolized by a dust generator. Sheep exposed for 30 minutes to 400 to 500 $\mu g/m^3$ of this aerosol did not show changes in pulmonary resistance, in static compliance, in airway reactivity to an aerosol of carbachol (a parasympathomimetic agent), or in tracheal mucus velocity (Abraham et al., 1980).

In another study, Chinese hamsters were exposed to 6,400 $\mu g/m^3$ for 6 months, 8 hours per day (Pepelko et al., 1979b). Exposed animals had conspicuously black lungs and increased lung weights. Vital capacity, residual volume, diffusion capacity for CO, and static deflation volume decreased. The findings were confirmed by histopathology, which indicated that possible emphysematous conditions had developed in the exposed animals.

Even so, a similar study with cats exposed for 1 year to approximately the same chamber atmosphere failed to reveal such deterioration in lung functions (Pepelko et al., 1979a). Paradoxically, animals had decreased closing volumes instead of that anticipated increases. In 25 rats exposed to 1,500 $\mu g/m^3$, 20 hours per day, 5-1/2 days per week, for 267 days, transpulmonary pressure, lung air flow, lung volume, and forced expiratory air flow were measured (Gross, 1979). No serious alterations in pulmonary functions were noted, although lung burdens of inhaled particulates were in excess of those to be expected in the general population.

Biochemical Effects

A limited number of studies have examined biochemical changes in the lungs of experimental animals exposed to diesel exhaust. In one study, a biochemical analysis of whole lung tissue was conducted by exposing rats for 3, 6, or 9 months to 250 or 1,500μ g/m^3 of diesel exhaust (Misiorowski *et al.*, 1979). Exposed animals had increased lung weights, total lung DNA, protein, and phospholipids. This was accomplished by an apparent increase of collagen biosynthesis in the highest dose group, although no net accumulation of pulmonary collagen was documented.

Other biochemical studies revealed a transitory stimulation of pulmonary prostaglandin dehydrogenase activity in guinea pigs exposed to a low dose of diesel exhaust. In animals exposed to 1,500 μg/m^3, enzyme activity decreased with time. It was not possible to demonstrate a similar phenomenon in rats because of the naturally low activity of this enzyme in the rat lung (Chaudhari *et al.*, 1979).

Rat and guinea pig lungs were also assayed for adenylate cyclase and guanylate cyclase following exposure to 250 or 1,500μ g/m^3 of diesel exhaust for up to 24 weeks (Schneider and Felt, 1979). Only guanylate cyclase seemed to decrease after diesel exhaust exposure, whereas adenylate cyclase was not affected. From these data, the investigators concluded that diesel exhaust exposure does not induce a tumor response. The authors of this report believe that such a conclusion cannot be sustained from the measurement of two pulmonary enzymes.

Finally, microsomal preparations from the lungs of rats exposed for up to 1 year to diesel exhaust had a decreased capability of metabolizing benzo[*a*]pyrene to polar metabolites. The reason for this was not clear. Whether diesel exhaust induced functional changes in microsomal preparations or inhibited some enzyme activity by its physical presence in the *in vitro* preparation was not resolved (Charboneau and McCauley, 1979). (See page 91.)

Extrapulmonary Effects

Systemic Organs

No information now indicates that exposure to diesel exhaust causes systemic toxic effects. In chronically exposed hamsters, some necrotic hepatic lesions were seen, as well as amyloidosis of the kidneys, the liver, the adrenal glands, and the spleen (U.S. DHEW, 1978). The changes were not considered to be related to particulate exposure because the incidence was similar in controls. Rats were exposed for 3 or 6 months to 6,000 $\mu g/m^3$ (Pereira *et al.*, 1979d) to test for the presence of abnormal *foci* in the liver. The results were negative, and no mention was made of abnormal liver pathology. Repeated intraperitoneal injections of diesel exhaust condensate in mice did not produce abnormal pathological changes (Orthoefer *et al.*, 1979).

In a long-term study with hamsters, several hematological parameters were measured. There were some slight changes in net hemoglobin and carboxyhemoglobin levels, but not in a range where serious risk and improper oxygenation of blood would be expected. Other hematological parameters were unaltered or only marginally changed (Heinrich *et al.*, 1979).

Yet the suggestion that inhalation of diesel exhaust may produce systemic effects is supported by the observation that a significant increase occurred in the activity of the enzyme, aryl hydrocarbon hydroxylase, in the prostate gland of rats after 2 weeks of exposure to a 1:13 dilution of diesel exhaust. Upon continuing the exposure for up to 6 weeks, aryl hydrocarbon hydroxylase activity was also found to be increased in the lungs and liver (Lee *et al.*, 1980).

Central Nervous System Effects

The effects of diesel exhaust exposure on functions of the central nervous system have been examined in two studies. Concentrations of diesel exhaust components in the exposure environment were: CO, 19.2 ppm; CO_2, 0.28 percent; hydrocarbons, 7.29 ppm; NO, 11.14 ppm; NO_2, 2.51 ppm; SO_2, 1.82 ppm; and particulates, 5.97 mg/m^3. Concentrations of these components in control air were 1.86 ppm, 0.05 percent, 3.22 ppm, 0.08 ppm, 0.03 ppm, 0.46 ppm, and 0.01 mg/m^3, respectively. Upon exposure, neonatal rats showed reduced spontaneous locomotor activity that persisted during adult life. Similarly, adult rats showed a decrease in spontaneous locomotor activity. In a subsequent experiment, sensory-evoked and/or visually evoked potentials in neonatal rats were measured following exposure. A significant difference between controls and exhaust-exposed animals was found. The data were interpreted to mean that diesel exhaust might influence the development of the central nervous system (Laurie and Boyes, 1979; Laurie *et al*., 1979).

Teratology

One study dealt with the effect of diesel exhaust on the fetal development of rabbits (U.S. EPA, 1979e). Twenty-one female rabbits were exposed to 10 percent diesel exhaust on days 6 to 18 of gestation for 18 hours per day. No maternal toxicity was seen in the exposed animals. The rabbits were sacrificed on day 29 of gestation and the fetuses were examined for external, visceral, and skeletal abnormalities. No malformations were found in a total of 170 fetuses examined. No significant differences between exposed and control groups were found in fertility, sex distribution, total number of fetuses, average fetal weight, and individual fetal weight.

Comparative Toxicities of Diesel and Gasoline Engine Exhausts

The toxicity of gasoline engine exhaust has recently been reviewed (Stupfel, 1976). Acute toxicity is largely due to CO. The demonstration of unequivocal chronic changes has usually proven to be more elusive. In dogs exposed for 18 months to natural and photochemically reacted gasoline engine exhaust, it was not possible to document changes in pulmonary function (Vaughan *et al*., 1969). In another long-term study with dogs, it was not possible to document hemodynamic abnormalities caused by the inhaled pollutants, although it was inferred that the CO present in the test atmosphere might have accelerated the development of arteriosclerotic lesions in a few animals (Bloch *et al*., 1972).

In rats, lifelong exposure to gasoline engine exhaust caused less weight gain and some neurobehavioral alterations but failed to reduce their lifespan or to produce signs of systemic toxicity; the development of emphysematous lesions in the lung appeared to be somewhat accelerated (Stupfel et al., 1973). Exposure to photochemically irradiated gasoline engine exhaust may increase susceptibility to pulmonary infection (Coffin and Blommer, 1967) or may produce more severe histopathological lesions in the lung (purulent bronchiolitis, pneumonia), as does exposure to exhaust from a gasoline engine without a catalytic converter (Stara et al., 1974). The effectiveness of the catalytic converter in reducing acute toxicity has also been demonstrated in rats (Lee et al., 1976).

More recent studies show that gasoline engine exhaust, whether or not treated by a catalytic converter, may induce subtle biochemical changes systemically or in the pulmonary system. In dogs exposed first to gasoline engine exhaust for 68 months and then to clean ambient air for another 32 to 36 months, the activity of prolyl hydroxylase in the lungs was significantly increased, although the ratio of collagen to total lung protein was unchanged (Orthoefer et al., 1976).

Detailed quantitative morphological studies showed that irreversible damage had developed, although concentrations of NO_x and O_3 within the chamber were below 2 ppm and 0.5 ppm, respectively, throughout the experiment. The most conspicuous changes were enlargement of proximal acinar air spaces and hyperplasia of the epithelium in the small airways (Hyde et al., 1978). Morphological changes in the airways and lung parenchyma thus developed at comparatively low levels of pollutants and appeared to be irreversible upon cessation of the exposure.

Lack of sufficient experimental data makes it impossible to provide an extensive quantitative comparison between pulmonary and systemic effects caused by exposure to diesel engine exhaust or to conventional gasoline engine exhaust. Overall toxicity appears to be low, although both have the potential to produce longlasting and irreversible lung lesions. The accumulation of pulmonary macrophages, so prominent in diesel exhaust-exposed animals, is due to the higher content of particulate material in diesel exhaust.

RESEARCH GAPS AND NEEDED RESEARCH

The data base on the pulmonary and systemic health effects of exposure to diesel exhaust is extremely limited. The following are the most obvious research gaps:

- There is a lack of information on the acute toxicity of diesel exhaust. A reevaluation of the acute effects of diesel exhaust on lungs should emphasize exposure to exhausts with different characteristics. These are generated by varying the modes of engine operation and by using different fuels. Primary lung damage and recovery should be fully documented with quantitative morphological techniques and selected physiological and biochemical studies (airway resistance, induction of protective enzymes, etc.). None of the presently conceived studies has considered the usefulness and value of detailed cell kinetic studies. These are of particular assistance in quantitating initial cell death in the lung (Evans _et_ _al_., 1978).
- It is necessary to determine the possible long-term consequences of diesel exhaust inhalation, such as the development of fibrotic and emphysematous changes in the lung. An important aspect would be to determine whether lesions are reversible upon cessation of exposure. In order to provide this information, different animal species should be exposed to graded concentrations of diesel exhaust in studies of several months duration, while fully documenting the extent and degree of the induced changes.
- Additional quantitative data need to be obtained on initial deposition and clearance of inhaled particulates, possible translocation to other organs and tissues, and retention in the body. The leaching of potentially toxic compounds from the particulates must be determined and related to potential systemic effects. A single study reporting that inhaled diesel exhaust causes biochemical changes in extrapulmonary tissue (Lee _et_ _al_., 1980) suggests the need for additional studies.
- Increased susceptibility of animals to infection following inhalation of diesel exhaust needs to be evaluated in young, mature, and old animals. A further question is whether resistance to a bacterial and/or viral challenge is modified primarily by the gas phase or by the particulate fraction of diesel exhaust. The functional biology of particulate-laden macrophages and the overall capacity of the macrophage system to handle

and remove particulate material under conditions of continuous exposure must be thoroughly investigated. This includes cell kinetic studies on the biology of macrophages (Adamson and Bowden, 1980) and quantitative morphometric studies. The effects of diesel exhaust on the immune system, in the lung, and in other organs must be evaluated with appropriate techniques (Vos, 1977).

- It is important to evaluate how diesel exhaust affects humans with preexisting diseases. For example, the presence of pulmonary emphysema has been shown in one study to alter the deposition and long-term clearance of inhaled particulates in hamsters (Hahn and Hobbs, 1979). That no experiments are planned with animals suffering from conditions similar to certain human diseases is clearly a research gap. Such animal models exist--e.g., pulmonary emphysema (Karlinsky and Snider, 1978), pulmonary fibrosis (Haschek and Witschi, 1979; Snider et al., 1978), immunosuppression from cigarette smoking (Holt et al., 1978), alveolar lipoproteinosis (Heppleston, 1975), chronic pulmonary hypertension (Kentera et al., 1978), increased sensitivity to ozone (Calabrese, 1978), and cardiomyopathy with general heart failure (Gertz, 1973). There is an urgent need to develop and use animal models of human diseases in order to relate the effects of specific primary and secondary products from diesel exhaust to the specific disorders present in humans.

CONCLUSIONS

Based on available information, few conclusions regarding pulmonary and systemic effects can be drawn. This is due to the paucity of information on human health and the preliminary state of the experimental work in progress.

- The acute and chronic inhalation of diesel exhaust produces, as expected, the accumulation in the deep lung of carbonaceous particulates as well as potentially hazardous compounds adsorbed to them. Such materials become sequestered primarily in alveolar macrophages and, to a limited extent, in cells of the alveolar epithelium. Clearance may occur via the mucociliary escalator and via the pulmonary lymphatic system. The possible long-term consequences of such accumulation with regard to its potential for causing chronic pulmonary disease are a key issue in the evaluation of diesel exhaust inhalation hazards. Furthermore, there is the question of whether adverse health effects may be exacerbated if synergistic interactions occurring in the environment (e.g., those between diesel exhaust particulates and products of photochemical reactions) increase the toxicity of exhaust components. Experimental data are insufficient to resolve this question.

- Histopathological changes induced by inhaled diesel exhaust are nonspecific. They may be interpreted to reflect initial cell damage followed by recovery with discrete areas of fibrosis and possibly emphysematous changes. The current data confirm that the fibrogenic potential of diesel exhaust is low. However, additional lifespan exposure data are needed.
- A single observation suggests that inhaled material may induce biochemical changes in organs distant from the respiratory tract. Because these materials are cleared relatively slowly, studies following inhalation exposure need to be of sufficient duration to determine the secondary effects of inhaled materials.
- Present information suggests that pulmonary defense mechanisms may be adversely affected by diesel exhaust. It is not clear whether the agents responsible for this phenomenon are associated with the gaseous or the particulate phase of the exhaust. Low levels of NO_x exposure have been shown to decrease resistance to infectious diseases in both animals and humans.
- Available evidence suggests that a single high-level exposure to diesel exhaust can produce acute toxic effects (e.g., poisoning due to NO_x, to aldehydes, and possibly to CO), whereas long-term exposure to comparatively low diesel exhaust levels has not clearly been shown to cause pulmonary and systemic toxicity. Determination of ultimate health effects requires consideration of the data bases on studies involving both acute and chronic exposures.
- Analysis of the available experimental evidence for pulmonary and/or systemic health effects caused by exposure to diesel exhaust suggests that it is possible to estimate the health hazards from the expected increase in gaseous and particulate components in the general atmosphere. With respect to pulmonary and systemic effects, it is reasonable to expect that the health hazards associated with certain pollutants originating in diesel exhaust (SO_x, NO_x, CO, and possibly particulate

material) would be qualitatively similar to those associated with the same pollutants from other sources (e.g., fossil-fueled power plants).

5 EPIDEMIOLOGY

Epidemiology is concerned with relationships between environmental exposures and disease frequency and distribution in clearly defined populations. The principal question is: What adverse effects can be anticipated from the increasing use of diesel-powered, light-duty vehicles on the health of the U.S. population during the next two decades? One way to answer the question is to monitor levels of and trends in exposure to diesel exhaust in different places and relate these to the levels of and trends in the health of the public. Studies of this kind are difficult to carry out successfully. It is difficult, for example, to anticipate all the other relevant changes that may occur over the next decade or more and to allow adequately for them. Moreover, unless it proves possible to identify a population living in an area where nearly a quarter of the light-duty vehicles are diesel-powered for comparison with one in which the proportion is much lower, no answer to the question could be obtained for 10 to 20 years, if then.

An alternative approach is to focus on population groups that have been exposed at work to high concentrations of diesel exhaust for long periods. The health of workers employed in bus garages, rail transport facilities, and underground mines where diesel engines are used can be compared with the health of workers at similar socioeconomic levels, in jobs that require comparable physical effort but do not involve exposure to diesel exhaust. If an adverse effect is found, it may be possible, by studying exposure-response relationships, to estimate the magnitude of effects on the working population. By making certain assumptions, it is also possible to estimate what the effects might be for the general population. One important proviso that should be considered is that there may be persons in the general population, but not in the working population, who are exceptionally susceptible to diesel exhaust emissions and therefore at greater risk than the general population.

Two critical questions about diesel exhaust that need to be addressed in epidemiological studies are:

- Does it cause cancer--more specifically, lung cancer?
- Does it cause chronic, nonmalignant respiratory disease?

If the answer to either of these questions is yes, the risk must be quantified by establishing exposure-response relationships.

REQUIREMENTS OF AN ADEQUATE RESEARCH PROGRAM

Cancer

The focus of research should be on respiratory cancer. Adequately designed studies of lung cancer will provide information about other sites in the body. Research on the risk of lung cancer from diesel exhaust is difficult because of the much greater known risk from cigarette smoking. It is important to consider adequately the extent of smoking before drawing firm conclusions about the risk from diesel exhaust. Research is made more difficult because of the long latency period between the first exposure to a carcinogen and the subsequent development of cancer. This means that if observations of the exposed population are begun now, there is little chance of obtaining a positive finding in fewer than 10 to 15 years. By contrast, a negative finding will be open to criticism unless the study covers perhaps 30 to 40 years. One way of avoiding such delay is to identify groups of people who have already been exposed to diesel exhaust for many years--preferably 20 years or more. Truck drivers, railway employees, garage workers, and underground workers in coal and other mineral mines are potentially suitable occupational groups for such studies. Unfortunately, although it may be possible to avoid the long wait to account for the cancer latency period by designating a study group (or cohort) that has been at risk for some years, other information about the group may not be available--or if available it is inadequately or inaccurately specified, leading to varying degrees of uncertainty and confidence. For instance, the tobacco-smoking habits of such a group are often unavailable, and measurements of exposure are likely to have been infrequent at best. It is sometimes possible to repair such deficiencies to some extent, particularly if a study that has defined a group in the past is to be continued into the future. Information on smoking behavior can be obtained retrospectively, using proxies for those who have died--though precise differences in intensity and inhalation levels will still be either unknown or uncertain.

Recognizing that epidemiological studies are complex and difficult to conduct, the panel suggests the following protocol for a study of health hazards from exposure to diesel exhaust. In such a study, the population group or cohort would be defined as all persons employed for 1 year or more in a specific occupation, going back to 1950, for instance. Personnel, union, and medical records would be used to categorize each employee by age, date of first employment, duration of employment, and estimate of exposure to diesel exhaust. It is likely that little or no information on smoking habits would be available from such records. Such information would have to be obtained from each employee in the group or from a proxy in the case of a worker who has died. This source might also be used to obtain information on other exposures before the worker entered the occupation or after leaving it.

Each member of the cohort would be followed to establish vital status. Death certificates would be obtained for those who have died, and causes of death would be categorized by a competent nosologist. Expected mortality for the U.S. or for the state or locality of the occupation group would be used for comparison with the mortality actually observed. It would be better if a comparable worker group not occupationally exposed to diesel exhaust would be identified, an identical follow-up carried out, and the findings compared for the diesel-exposed and nondiesel-exposed populations. Comparisons of risk would be made in employees exposed to different doses of diesel exhaust. The follow-up period should be at least 20 years--preferably 30 years or more--because of the long latency period for cancer. This may mean that a study begun in the past would continue well into the future. Continuing a study in this way provides an opportunity for collecting good data on current exposures, thus filling the gaps to some extent in this aspect of the study.

Information on cancer morbidity is harder to obtain than information on mortality. This is largely immaterial for lung cancer because most cases are fatal and run a relatively short course. Information on cancer incidence is desirable for other cancer sites, however, and morbidity data would therefore be desirable. If a study is conducted in an area served by a good cancer registry, it may be possible to obtain information on cancer incidence as well as cancer mortality. The state of Connecticut is particularly suitable for a study of this kind because it has had an excellent cancer registry since 1935 (Connecticut State Department of Health, 1968).

Apart from a forward-looking (or prospective) study of the type just described, in which the occurrence of lung cancer is related to previous exposure, conclusions about the impact of diesel exhaust on lung cancer can also be drawn from a comparison of the experience of lung cancer patients with that of other people. In a study of this kind, persons who have developed lung cancer or who died of the malignancy would be identified. Then a suitable control group would be selected for comparison in an acceptable way. Ideally, this group would be representative of the population from which the lung cancer cases have come. Often, it is desirable to match the comparable group, for example, with respect to age and sex. Exposure to diesel exhaust of cases and controls would need to be ascertained.

Essentially, a study of this kind aims to find out whether people with lung cancer have been more heavily exposed to diesel exhaust than others, and, if so, how much more. It is also necessary to consider other factors that are known to influence the occurrence of lung cancer before drawing conclusions about the effect of diesel exhaust. Thus, occupational exposures known to cause lung cancer, as well as such factors as smoking habits, residence in polluted areas, exposure to certain hazardous consumer products in and around the home, and socioeconomic status, need to be recorded for cases and controls in order to properly evaluate the possible cause-and-effect relationships.

Backward-looking (or retrospective) studies of this kind can be applied to other diseases--for example, chronic nonmalignant respiratory diseases, which are thought to be influenced by exposure to diesel exhaust. To some extent, such studies complement the forward-looking study described above. It may be harder to draw valid inferences from backward-looking studies than from forward-looking studies, although they have the advantage that they can be carried out in a relatively short time. When the conclusions of both types of studies are in agreement, there is greater confidence in their validity.

Nonmalignant Chronic Respiratory Diseases

An adequate study of nonmaligant chronic respiratory diseases would normally be rather different from a study of cancer. Initially such an effort might comprise a cross-sectional study in which workers exposed to diesel exhaust are compared with similar workers who are not so exposed. Both groups would be examined over a similar short time. Questionnaires would be completed about respiratory symptoms, current and past chest illnesses, smoking habits, occupational and residential exposures, and detailed information on exposure to diesel exhaust. The study would include spirometry, using simple, well-tested methods of evaluating lung function, and possibly chest radiography. Standardized methods of obtaining this information have been recommended by the National Heart, Lung, and Blood Institute, together with the precautions that need to be taken to ensure the validity of the results (Ferris, 1978). The data would be analyzed to compare the prevalence of respiratory symptoms and level of ventilatory lung function in exposed and nonexposed workers, and among the exposed according to the level of exposure, after making due allowance for the effects of other variables such as age, sex, smoking, and other factors.

In addition to observations made at a single point in time, it would also be helpful to have serial observations made over a number of years. In this way, the rate of development or of remission of symptoms and illnesses, the changes in the level of lung function, and the outcome (or mortality) can be related to diesel exhaust.

EVALUATION OF COMPLETED STUDIES

Lung Cancer

General Trends

The belief that air pollution is an important factor in the causation of lung cancer has been based on early observations that mortality rates were higher in urban than in rural areas, coupled with the knowledge that carcinogens were present in polluted urban air. Support for this came from the findings in England and in Wales that there was a positive correlation between smoke concentrations and lung cancer mortality rates in different localities. Later studies, however, showed that this correlation had been exaggerated by the selection of extreme cases (Buck and Brown, 1964; Waller, 1967). When towns of similar size were compared, little evidence could be shown of any association between pollutant concentrations and lung cancer mortality. It has now become clear that the urban/rural gradient in lung cancer mortality, which was first thought to be due to differences in air pollution, is mainly due to other matters--notably cigarette smoking, occupational exposures, opportunity for medical care, and various socioeconomic factors. In particular, the dominant role of cigarette smoking in the etiology of lung cancer makes it hard to assess the minor contribution of air pollution.

The finding that British migrants to New Zealand, to South Africa, and to Australia had higher death rates from lung cancer than native-born New Zealanders, South Africans, or Australians, and particularly the observation that the rates were higher in those migrants, aged 30 years and over, who left Britain than in those who left at younger ages, despite similar smoking habits, suggests the influence of some environmental factor, possibly pollution associated with early life in Britain. It is now well-recognized that migrants are not representative of a native population. Nor has adequate attention been given to the jobs they take after migrating. Moreover, mobility appears to be related in some way to the development of lung cancer (Haenszel *et al*., 1962, 1964).

The effect of diesel exhaust pollution on the general population is even harder to assess. The report of the British Royal College of Physicians on *Air*

Pollution and Health (1970) points out that the increase in the use of diesel fuel in Britain followed the striking rise in mortality from lung cancer in England and Wales, and therefore could not have played a major role in the etiology of that disease. During the past 10 to 15 years in England and Wales, respiratory cancer rates have been declining in men under 65 years, but increasing among women. These trends cannot readily be attributed to trends in diesel exhaust, but they follow quite understandably from known trends in cigarette smoking. (See Figure 5.1).

Transportation Workers

There have been a number of suggestions that persons who might have been exposed at work to polycyclic organic matter from diesel exhaust had elevated cancer risks. Thus, Hueper (1955) noted high rates for cancer of the lung in transportation workers exposed to exhausts from gasoline and diesel engines, to petroleum lubricants, and to dust from asphalt roads. He found that on the basis of crude, nonstandardized (sex, age) figures, about 75 percent of the lung cancers occurred among railroad employees, who constituted 25 percent of the total number of employees. The source of this evidence, which would be more impressive had it been standardized, is not altogether clear, and no information is given on how it was collected. While the observation points to needed research, no conclusions can be drawn from it.

Finnish Railroad Workers

Hannunkari and coworkers (1978) have related working conditions to mortality and disability among railroad workers in Finland. Engineers, trainmen, and clerks 30 to 52 years of age were compared. Rates were found to be higher at all ages among the engineers, with malignant tumor rates significantly higher at the 5 percent level. It is not clear from the paper to what extent the three groups differed with respect to diesel exhaust exposure, and no allowance was made for other potentially confounding variables such as smoking or socioeconomic status.

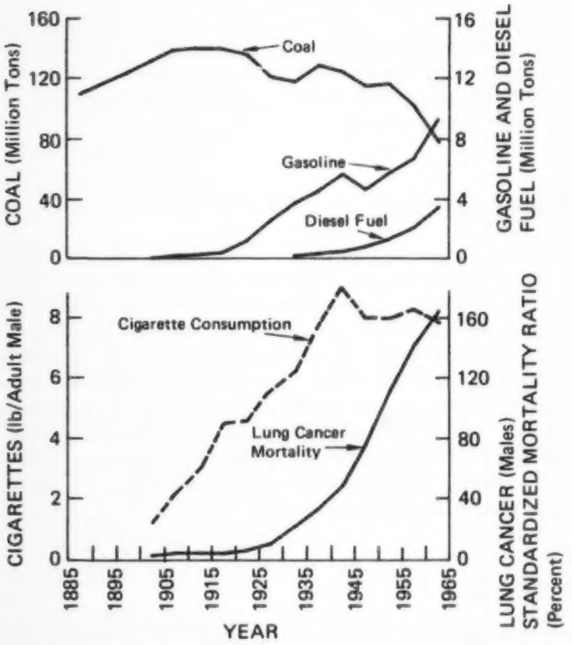

FIGURE 5.1 Time trends in fuel consumption, cigarette smoking, and excess mortality[a] from lung cancer in males. (Excess mortality is the difference between the expected and the actual number of deaths.) (Source: Royal College of Physicians, 1970.)

Occupationally Related Diseases of Persons Admitted to the Roswell Park Memorial Institute

Decoufle and coworkers (1977) have analyzed the occupations of 24,416 people admitted to the Roswell Park Memorial Institute between 1956 and 1965. Information about smoking and occupation had been obtained before the diagnosis was made. Persons without cancer were used as controls. Occupation was categorized, either as any duration of employment in specific jobs or employment for at least 5 years or more. There was no significant increase in the relative risk of cancer at any site for railroad engineers or firemen. Truck and tractor drivers had increased risks for colon and rectal cancer. Increased relative risks of buccal cavity, pharynx, and urinary bladder cancers among engineers and firemen, as well as of malignant lymphoma, did not reach conventional statistical significance.

Occupational Groups in Los Angeles County

In a study of occupational factors related to lung cancer using data from the Los Angeles County Cancer Surveillance Program, Menck and Henderson (1976) reported an increase among truck drivers (standard mortality ratio 165), auto repair workers (standard mortality ratio 146), and transportation workers (standard mortality ratio 127). No allowance was made for smoking. Interpretation of these modestly raised rates is uncertain. Moreover, these occupational groups are exposed to many substances other than those identified in diesel exhaust that could influence their liability to cancer.

U.S. Coal Miners

The most comprehensive study of mortality among U.S. coal miners was carried out by Rockette (1977). The study followed 22,998 miners, covered by the United Mine Workers of America health and retirement fund, between January 1959 and December 1971. The aim of the study was "...to determine whether or not coal miners have an excess mortality when compared to the total U.S. population." During the study period, 7,628 deaths occurred. The mortality for these coal miners compared with that expected for the U.S. male population, 1959-1971, is shown in Table 5.1. A modest elevation of the standard mortality ratio for respiratory cancer is seen (112.5). Rockette has noted that the elevation was "...well within the range of what might result from differences in residence or smoking habits of the control and study groups."

The effect of exposures to diesel exhaust was not addressed in this study. It is possible to use the data to evaluate such exposures. Of all dieselized coal mines in the U.S., 95 percent are in an area covering the states of Washington, Montana, Wyoming, Utah, Colorado, New Mexico, and Alaska (American Mining Congress, 1978).

TABLE 5.1 Observed and Expected Deaths and Standardized Mortality Ratios for Selected Causes Among U.S. Coal Miners

Cause of Death	Observed	Expected	Standardized Mortality Ratio
All causes	7,628	7,506.1	107.6
All cancer	1,223	1,252.2	97.7
Respiratory cancer	373	331.5	112.5[a]
Chronic and unqualified bronchitis	26	31.5	82.5[a]
Emphysema	170	118.3	143.7[a]
Asthma	32	18.3	174.9[a]
Accidents	408	283.0	144.2[a]
Ill-defined	162	86.2	187.9[a]
All other	625	459.5	136.0[a]

[a] Significantly different from expected at 5 percent level.

Source: Adapted from Rockette (1977).

Of all 10 coal mining districts, this district had the lowest death rates from all causes, from all cancers, and from cardiovascular disease; the second lowest from respiratory cancer; and the third lowest from digestive system cancer. In contrast, it has the second highest standard mortality ratio for emphysema.

This evidence does not suggest that exposure to diesel exhaust in U.S. coal mines poses a hazard for lung cancer or other cancers, although it is possible that the time since first exposure may not have been long enough for cancer detection. It may, however, suggest that such emissions pose a hazard for emphysema and clearly indicates a need for further research.

Baltimore and Ohio Railroad Workers

Kaplan (1959) has reviewed the medical records of the Baltimore and Ohio Railroad Relief Department. Membership and mortality records were tabulated from January 1953 to December 1958. Deaths from all causes, including lung cancer, were compared with those expected on the basis of death rates for the general population, as estimated by the American Cancer Society. Railroad workers were then categorized into three groups according to their liability to diesel exhaust exposure:

- Group 1 (operating) consisted of employees directly exposed to diesel exhaust. It included engineers, firemen, motormen, road brakemen, yard helpers, yard foremen, conductors, switchmen, yard masters, and train masters.
- Group 2 (nonoperating) included employees who performed manual labor or mechanical services in shops or roundhouses. They are exposed to a lesser degree to pollution from diesel engines, coal smoke, soots, dust, lubricating oil, and welding fumes. This group included carmen, carmen helpers, electricians, blacksmiths, boilermakers, machinists, pipefitters, molders, painters, carpenters, trackmen, patrolmen, and dump operators.
- Group 3 (nonoperating) consisted of persons who are rarely exposed to noxious fumes during their work. These included clerks, laborers, janitors, agents, bridge inspectors, and port captains.

The observed and expected numbers of deaths from lung cancer for each of these groups from 1953 to 1958 are shown in Table 5.2. The results show no clear relationship between potentially noxious exposures and mortality. Overall, the rates were fractionally higher in group 3 than in group 1 and appreciably higher in both these groups than in group 2.

Smoking habits were not considered, and the duration of exposure was probably not long enough to allow for the latency of cancer. Dieselization of the Baltimore and Ohio Railroad began in 1935 and was not completed until 1958, the last year of the study. Thus, the maximum exposure time was 23 years, and the average exposure time of the heavily exposed group may well have been less than 10 years. Because of such short exposure periods, caution should be observed in drawing conclusions from this study.

London Transport Workers

Raffle (1957) reported the lung cancer incidence among male employees of the London Transport Authority, aged 45 to 64, during 1950 to 1954. The report dealt mainly with the general problem of occupational health, and the findings on lung cancer were only briefly noted. On the basis of a review of medical records, lung cancer deaths occurring during service were ascertained, as well as lung cancer cases occurring at the time of retirement or

TABLE 5.2 Expected and Actual Number of Deaths from Lung Cancer Among Employees of the Baltimore and Ohio Railroad Company, 1953-58[a]

	Group 1[b]			Group 2[b]			Group 3[b]			Total All Groups		
		Deaths			Deaths			Deaths			Deaths	
Year	Members[c]	Expected	Actual	Members[c]	Expected	Actual	Members[c]	Expected	Actual	Members[c]	Expected	Actual
1953	13,210	8.2	8	22,930	13.6	12	7,680	6.5	3	43,820	28.3	23
1954	12,360	8.6	6	21,350	14.3	14	7,260	6.7	9	40,970	29.5	29
1955	12,160	8.9	3	20,380	15.0	11	6,890	6.9	4	39,430	30.8	18
1956	12,470	9.9	8	19,820	16.6	11	6,660	7.3	10	38,950	33.8	29
1957[d]	12,250	10.3	13	18,790	17.2	8	6,410	7.5	7	37,450	35.0	28
1958[d]	11,340	10.2	11	17,200	16.8	11	5,950	7.6	5	34,490	34.6	27
6-year total	73,790	55.9	49	120,470	93.5	67	40,850	42.5	38	235,110	192.0	154
Percent of actual to expected death rate per 100,000	--	87.5	--	--	71.6	--	--	89.4	--	--	80.2	--
National rate per 100,000	--	75.9	--	--	77.6	--	--	104.1	--	--	81.7	--
Relief Dept. rate per 100,000	--	66.4	--	--	55.6	--	--	93.0	--	--	65.5	--

[a] Based on standardized national death rates and annual Baltimore and Ohio Railroad Company Relief Department exposures for each occupational group.

[b] Group 1--employees directly exposed to diesel exhaust; Group 2--employees who were exposed to a lesser degree; Group 3--employees who were rarely exposed.

[c] Average number of members participating in the Baltimore and Ohio Railroad Relief Department program.

[d] Estimated national death rates furnished by American Cancer Society.

Source: Adapted from Kaplan (1959).

transfer to alternative work within the London Transport Authority. Lung cancer cases occurring after leaving the London Transport Authority were not recorded. The basic assumption of this analysis was that mechanics (or engineering staff) working in bus garages were exposed to an excess of diesel exhaust, and that if such exhaust caused lung cancer, then they would experience a higher incidence than that of the general population and of other male employees of the London Transport Authority.

The analysis is shown in Table 5.3. The categories of London Transport Authority staff are presented in order of presumed increasing exposure to diesel exhaust (motormen and guards on the underground railways generally having the lowest exposures and engineering staff in bus garages having the highest). The reported death rates in Table 5.3 were not age-adjusted. There is no clear relationship between the presumed degree of exposure and the annual lung cancer incidence. The rates in the most heavily exposed groups do not appear to exceed those for men of comparable age in England and Wales. Raffle remarked, however, that "...both the bus and trolleybus mechanics were slightly older than men of England and Wales, while the age distributions of the other job categories were not significantly different."

Raffle's original study has been updated by Waller (1979) to cover the period from 1950 to 1974. Except for the extended period of observation, the basic assumptions and methods of observation are the same. Lung cancer cases and man-years at risk for five job categories are shown in Table 5.4. The lung cancer incidence among all London Transport Authority employees was 79 percent of what could be expected from lung cancer mortality rates of men the same age in Greater London. No consistent dose-related trend emerges from the data. The highest standard mortality ratio (0.90 compared with Greater London rates) was among garage mechanics, who presumably had the highest exposure to diesel exhaust. The second highest standard mortality ratio, however, was among motormen and guards, who presumably did not suffer excess exposure to diesel exhaust.

Both studies, the original and the update, suffer from many weaknesses in design. Although some measurements of whole smoke and polyaromatic hydrocarbon concentrations were taken in selected bus garages, individual exposures were not measured. The presumed exposure rankings in Table 5.3 and 5.4 were based

TABLE 5.3 Deaths, Ill-health Retirements, and Transfers to Alternative Work due to Lung Cancer in London Transport Authority Male Staff Aged 45-64

Group or Staff	Man-years at Risk	Deaths, Ill-health Retirements, and Transfers to Alternative Work: 1950-54	
		Number[a]	Annual Rate per 1,000
Motormen and guards (London Transport Railways)	8,253	11 (10)	1.3
Engineering staff (Trolley Bus Depots)	5,529	10 (9)	1.8
Engineering staff in bus garages (Chiswick Works)	9,979	12 (12)	1.2
Central bus drivers	33,466	23 (17)	0.7
Central bus conductors	16,978	18 (15)	1.1
Engineering staff	18,140	22 (21)	1.2
Population	Male Population[b] Aged 45-64	Deaths 1950-53	
		Number	Annual Rate per 1,000
England and Wales	19,947,000	26,689	1.3
Greater London	3,834,000	6,292	1.6

[a] Deaths are in parentheses.
[b] 1950-53.

Source: Raffle (1957).

primarily on general observations, and quantitative differences in the degree of exposure are uncertain. Smoking habits were not measured. Because even small differences in smoking rates would have a significant effect on lung cancer incidence, comparisons among categories are subject to further uncertainty. Differences in smoking habits might also have contributed to the discrepancies between all London Transport Authority workers and men residing in Greater London. The generally lower lung cancer rates among male employees of the London Transport Authority may also reflect a "healthy worker effect," or higher

TABLE 5.4 Lung Cancer Cases Among London Transport Authority Staff in Relation to the Number Expected on the Basis of Greater London Death-Rates (1950-74, males aged 45-64 only)

Job Category	Man-years at Risk	Expected Deaths	Observed Cases	Mortality Ratio (%)
Motormen and guards	35,610	67.7	59	87
Engineers (Central Works)	30,031	63.2	42	66
Bus drivers	175,909	346.8	259	75
Bus conductors	93,095	174.5	130	75
Engineers, garages	86,054	197.1	177	90
Total	420,699	849.2	667	79

Source: Waller (1979).

socioeconomic status of these men compared with men from England and Wales or Greater London. Failure to follow up employees who had left the organization may also have contributed to this discrepancy.

In Raffle's original study, the number of lung cancer cases may have been too small to ascertain a significant effect from diesel exhaust exposure. Moreover, the time from initial exposure to diesel exhaust to the end of the observation period was likely to have been too short to produce a detectable effect. Waller's extension from 1950 to 1974 has the potential to resolve these weaknesses. But data on the average length of employment in any of the job categories is lacking.

There is no real evidence from the London Transport study that excess risk of lung cancer is associated with exposure to diesel exhaust. It seems clear that such exposure was not an important cause of lung cancer in this population. The many shortcomings of the study make it necessary, however, to view these conclusions with caution. Waller (1979) has suggested that a cohort study of these workers may be in the offing. Such a study should produce much more definitive data, particularly if allowance can be made for smoking.

In a more detailed analysis of the trends in lung cancer among London Transport Authority job categories during the periods 1950 to 1960 and 1961 to 1974, Harris (1981) has determined that the risk of lung cancer among the most exposed group (the garage mechanics) was not significantly different from that of the other London Transport Authority workers. Harris also used the upper and lower bounds of the estimated lung cancer risk of the London Transport Authority garage mechanics to determine the potential range of risk of lung cancer associated with future ambient exposure to diesel engine exhaust in the United States.

Potash Workers

A study of potash miners conducted by Waxweiler and coworkers (1973) provides some observations on the relation of exposure to diesel emissions and lung cancer. The study was conducted to determine if the higher risk of lung cancer among underground ore miners could be attributed to any extent to a constitutional predisposition in those who chose to work underground. Potash miners were selected because potash ore is not embedded in siliceous rock, the level of radon in the air is not significantly higher than in the ambient air, and other lung cancer-causing agents such as arsenic, nickel, cobalt, and chromium are present in very low concentrations--if at all.

The study covered miners and millers from 8 companies, who had worked for at least 1 year between January 1940 and July 1967. Mortality up to 1967 was compared with that expected for the total U.S. population. Diesel engines had been used for transportation in 2 mines, in one for 18 years and in the other for 10 years. Miners who worked in diesel and nondiesel mines were compared. The authors reported that there was no significant difference in the causes of death between the 2 groups. Although they reported 31 deaths in 6,733 person-years in the 2 diesel mines, no details were given to support the finding. They also noted that the elapsed time may have been insufficient for chronic diseases or those diseases with long latency periods to show up. This was a small study, and diesel engines had not been in the mines for very long. It would be unwise, therefore, to draw any conclusions on the relation of diesel exhaust to lung cancer.

Truck Drivers

In a study of mortality among unionized truck drivers, Leupker and Smith (1978) have compared the experience of teamsters with that of the general population of the U.S. They noted a statistically significant increase in death rates from cancer of the respiratory tract among teamsters, amounting to 37 percent in the 50 to 59 age group. Although the higher incidence rate might be due to exposure to diesel exhaust, no evidence was presented to support a greater degree of exposure among teamsters than among the general population. Many other differences may exist between this occupational group and the general population. In the absence of such essential information as geographical distribution, urban-rural balance, smoking habits, and socioeconomic circumstances, it is impossible to draw reliable conclusions from this study.

Nonmalignant Respiratory Diseases

There have been a number of studies of the prevalence of respiratory symptoms and the level of ventilatory lung function, in which persons exposed to diesel smoke at work have been compared with others who were not exposed.

Locomotive Repairmen

Battigelli and coworkers (1964) have carried out a clinical and physiological comparison of 210 locomotive repairmen exposed to diesel exhaust. The control group included 154 railroad workers comparable in age, body size, occupational activity, and smoking habits, who were not exposed to diesel exhaust. No differences in symptom prevalence or level of lung function could be attributed to the diesel exhaust exposure, but there was a clear effect of smoking on both these indices.

Coal Miners

Reger and Hancock (undated) have compared underground coal miners exposed to diesel exhaust with a control group of underground miners who were not exposed. Matching and comparison were based on the Interagency

Study of Coal Workers' Pneumoconiosis. The exposed miners were found to have a significantly higher prevalence of persistent cough and sputum and a lower lung function than those who were not exposed. Conversely, the exposed miners experienced a lower prevalence of wheezing and dyspnea. It is not easy to interpret the conflicting findings. Lower levels of lung function among the exposed workers suggest that diesel exhaust may cause a chronic respiratory effect. The comparison was based on examination of the miners exposed to diesel exhaust at a later date than the control miners. This could have introduced bias into the findings. In a further study of lung function changes before and after a working shift, Reger and coworkers (undated) showed that, although lung function declined during the day, there was no significant difference between those exposed and those not exposed to diesel exhaust.

Underground Metal and Nonmetal Miners

During 1976 and 1977 the National Institute for Occupational Safety and Health conducted a study of respiratory health in men employed in 21 metal and nonmetal mines (Attfield, 1978). The purpose was to evaluate the effect of exposure to silica dust and diesel exhaust. The findings with respect to diesel exhaust exposure were inconsistent. An increased prevalence of persistent cough was associated with exposure to aldehydes. However, there was no concomitant reduction in lung function. No consistent changes in symptom prevalence or level of lung function were attributable to exposure to nitrogen dioxide. Although the prevalence of persistent cough and breathlessness was slightly higher in those who had been exposed for 5 years or more to diesel exhaust than in those who had been exposed for fewer than 5 years, lung function also tended to be better in those with the longest exposure. In short, the results did not suggest that exposure to diesel exhaust contributed to a significant chronic respiratory disease in this population.

Iron Ore Miners

Jorgensen and Svensson (1970) have compared 120 underground iron ore miners with 120 surface miners in 149 Sweden. Increasing use of diesel vehicles for underground transportation since 1959 had led to potential exposures to nitrogen dioxide of orders ranging from 0.5 to 1.5 ppm The two groups of workers were balanced for age (younger and older groups) and smoking (smokers and nonsmokers). Respiratory symptoms were recorded, using the British Medical Research Council's questionnaire, and lung function was assessed by spirometry. Bronchitis, defined as productive cough lasting 3 weeks or more for several successive winters, was more prevalent in underground workers than in surface workers, in older than in younger workers, and especially in smokers compared with nonsmokers. In contrast, there were no differences in lung function other than those due to age. Lung function values in smokers were no lower than in nonsmokers and in underground workers were no lower than in surface workers.

It is difficult to draw conclusions about the effect of diesel exhaust from this study. Comparison of surface with underground workers is not satisfactory because it fails to separate differences, other than diesel exhaust exposure, that may exist in the underground environment. The reasons why a man chooses to work on the surface or underground may also be relevant and require consideration.

Railway Workers

A study of ventilatory lung function among 475 Japanese railway workers exposed to diesel smoke was described by Yamazaki (1969). The subjects were members of the Railway Labor Research Institute who worked at a number of sites in tunnels and railway inspection and repair sheds. The 67 control subjects included 37 office workers and 28 members of the Railway Labor Research Institute. The findings were analyzed using the method of quantification theory described by Hayashi (1961). In the analysis, 11 items that might be related to pulmonary function were divided into 36 categories. Age, height, and weight were found to have the greatest effect on lung function. Factors related to employment (employment site, level of pollution by exhaust gas, and job designation) were next in importance, and they apparently exerted a greater effect than smoking. The authors concluded, however, that the work site factor would reduce lung function by 10 percent at most, which they did not consider to be high.

Workers in Bus Garages in Egypt

El Batawi and Noweir (1966) have conducted a study of 161 employees in 2 diesel bus garages in Alexandria, Egypt. The air in the garages was monitored over 3 winter months. Sulfur dioxide, nitrogen dioxide, hydrocarbons, aldehydes (e.g., acrolein), and soot were measured. The concentrations of these pollutants were below known threshold values. However, the authors concluded that because eye tearing and nasal irritation were evident during visits to the garages, acrolein concentrations may have been high.

The prevalence of respiratory diseases was high relative to the prevalence in occupational groups without such environmental exposure studied previously by the authors. Upper respiratory tract disease, chronic bronchitis, and asthma were higher by factors of 1.7, 1.6, and 3.9, respectively. Peptic ulcer, gastritis, and high blood pressure were also noted to be in excess among exposed employees. Smoking, which was not accounted for in this study, could be responsible for some or all of the higher incidence rates. Among the workers, 72 percent were heavy smokers. A much more carefully controlled study is considered necessary to clarify the relationship between diesel exhaust exposure and the observed respiratory effects.

EVALUATION OF PLANNED STUDIES

Two major epidemiological investigations of diesel exhaust exposure are currently planned. The first (Milby et al., 1979) is a study of heavy equipment operators in the Operating Engineers Union. A cohort is to be defined from union records, making eligible any man who has worked for 1 year or more between January 1958 and December 1978. It is estimated that the cohort will be composed of 25,000 to 40,000 subjects with up to 500,000 man-years of work experience. Name, address, social security number, birth date, and past and present jobs held (with the total number of hours in each job) are said to be available. Thus, some classification with respect to exposure should be possible. Follow up of mortality is envisaged using membership records, pensioner records from the union's Health and Welfare Plan, and information from the Social Security Administration. Additional follow-up methods are also mentioned for particularly difficult cases. These include the use of commercial organizations that are effective, although expensive. Classification of exposures will be based on the time and intensity of exposures. Comprehensive studies of industrial hygiene will be made in order to obtain good evidence of the exposure intensity.

In addition to studying mortality in the whole cohort, a study of mortality among union pensioners is also planned. Comparison of worker mortality will be made with standard life expectancy tables for males in the United States and in the state of California. In addition to mortality, the proposal calls for a study of cancer incidence, using a computer-matching system that will enable a comparison of cancer incidence in workers exposed to diesel exhaust and those not exposed. Also under consideration is a cross-sectional survey of chronic pulmonary disease among workers exposed to diesel exhaust.

The plan appears to have been well-conceived and in close agreement with the suggestions outlined earlier in this report. Its success depends on the intensity of the exposures to which this cohort of workers has been exposed in the past and is still being exposed. The evidence presented is somewhat discouraging. Conclusions from measurements made to date suggest that the diesel equipment has made little contribution to carbon monoxide and respirable particles. The contribution to nitric oxide and nitrogen dioxide was to raise concentrations of these chemicals significantly above background only when other vehicles were immediately upwind.

The second proposal (Schenker and Speizer, 1979) is a retrospective study of railroad workers exposed to diesel exhaust. The investigators plan to use Railroad Retirement Board records to identify approximately 80,000 subjects (including those with the longest and heaviest diesel exhaust exposures) who had from 10 to 19 years of service as railroad employees in 1964. The cohort will be followed through 1978. The causes of all deaths during the working years and after retirement will be ascertained from death certificates. It is calculated that 2,483 deaths will have occurred from lung cancer. This should be sufficient to demonstrate a 1.5 relative risk of lung cancer. (It is not clear how smoking habits will be ascertained in this cohort.) In addition, the investigators plan to study approximately 300 cases of death due to lung cancer, 300 age- and sex-matched deaths due to nonrespiratory cancer, and 300 nonmalignant, nonaccidental deaths. Information on potentially complicating factors, such as cigarette smoking and residential differences (urban versus rural), will be collected by means of questionnaires sent to the immediate families. Hospital records, including reviews of pathology, will be requested for lung cancer cases. These efforts will be supplemented with an integration into the cohort selection and data analysis of environmental and personal monitoring of levels of diesel exhaust exposure for different job categories and work areas. These research workers believe that they can incorporate information on smoking, as well as diesel exhaust exposures in different places and at different times, based on environmental measurements and personnel samplers from their case/control study, into their large cohort study, while making due allowance for major complicating factors. It is not clear from the available study proposal how this can be done.

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

The following conclusions can be appropriately drawn from the review of the literature on diesel exhaust exposures:

- In epidemiological studies of occupational exposure to diesel engine emissions, excess risk of cancer of the lung, or of any other site, has not been convincingly demonstrated. The evidence to date does not indicate that exposure to diesel exhaust is a serious cancer hazard, at least at exposure levels no greater than those that existed in London bus garages. Only two studies, one on railroad workers (Kaplan, 1959) and the other on bus garage workers (Raffle, 1957; Waller, 1979), approximate even the minimum requirements for a sound epidemiological evaluation of cancer risk. Both of these studies, however, suffer from numerous deficiencies in design. Hence, their negative conclusions must be viewed with caution.
- Evidence of a relation between occupational exposure to diesel exhaust and the prevalence of chronic obstructive lung disease is inconsistent. Some studies have suggested that workers exposed to diesel exhaust have a higher prevalence of chronic respiratory symptoms, bronchitis, and diminished lung function than otherwise

comparable persons who have not been exposed. Other studies have failed to confirm these observations.

- Additional carefully controlled studies of populations occupationally exposed to diesel engine exhaust are needed. In these studies, both the whole exhaust and its individual components should be carefully monitored. These studies must carefully control for cigarette smoking, which plays a dominant role in the etiologies of lung cancer and chronic obstructive lung disease.
- Several epidemiological studies have suggested synergism between cigarette smoking and occupational exposure in the development of lung cancer (International Union Against Cancer, 1976). Asbestos workers and uranium miners who smoke appear to be exceptionally prone to develop this cancer (Archer *et al*., 1973; Selikoff, *et al*., 1968, 1980). Synergism between smoking and diesel exhaust might similarly increase the risk of lung cancer, although this has not yet been shown to occur. Future epidemiological studies should keep this possibility in mind.

GLOSSARY

acinar air spaces - Air spaces within the basic functional unit of the lung, called the acinus, which consists of respiratory bronchioles, alveolar ducts, and alveolar sacs.

adenoma - A benign tumor of the epithelium (see definition).

adenylate cyclase - An enzyme that catalyzes the formation of cyclic AMP (adenosine monophosphate).

alkylating agent - A compound that transfers an alkyl group such as a methyl or ethyl ($-CH_3$ or $-C_2H_5$) to an organic molecule. The process is called alkylation.

alveolar septal hyperplasia - An abnormal increase in the number of cells in the walls of the alveoli in the lungs.

amines - Organic compounds that can be thought of as derived by substituting organic radicals (R-) for one or more of the H's on ammonia (NH_3): for example, $R-NH_2$, a primary amine; R_2NH, a secondary amine; and R_3N, a tertiary amine.

methylarylamine: in o-methylarylamine, the R- is an aromatic hydrocarbon (see definition) and there is a $-CH_3$ (methyl) side-group bonded to the carbon atom immediately adjacent to the amine.

nitrosamine: In a nitrosamine, an -NO (nitroso) group is substituted for the H of a secondary amine, e.g., R_2N-NO.

amyloidosis - The accumulation of amyloid (an abnormal proteinaceous material) in various body tissues.

arene - A hydrocarbon containing at least one 6-membered benzene (C_6H_6) ring.

arene oxide: An arene oxide has oxygen atoms attached to the central aromatic nucleus. The simplest, quinone, has two oxygen atoms (O) attached to two different carbon atoms (C) on a benzene ring.

nitroarene: A nitroarene has one or more $-NO_2$ (nitro) groups attached to the aromatic nucleus.

aromatic hydrocarbon - An organic compound containing one or more 6-membered benzene rings.

aryl hydrocarbon hydroxylase - An enzyme that catalyzes the attachment of an hydroxyl (-OH) group to hydrocarbons.

base-pair substitution mutation - See mutation.

bioavailability - In the context of this report, the availability for biological processing and interaction of materials attached to the carbonaceous core of diesel exhaust particulates.

bronchiolization - An overlaying of alveolar epithelium with cells that are characteristic of bronchial epithelium.

carcinogen - Any cancer-producing substance.

carcinoma - A malignant new growth, composed of cells of the epithelium (see definition), which tends to infiltrate the surrounding tissues and which gives rise to metastasis (the transfer of malignant cells to another part of the body).

cardiomyopathy - A disorder or disease of heart muscle.

cellular atypia - Irregular or nonconforming cells, often indicative of precancerous changes.

chemical pneumonitis - A condition of localized acute inflammation of the lungs caused by a chemical agent.

clastogen - A substance that causes chromosome breakage.

cocarcinogen - Any noncarcinogenic agent that will enhance the tumor response induced by a carcinogen (sometimes used to refer to those agents that will do so only when applied simultaneously with the carcinogen).

cohort - A defined group in a study that is followed up over time.

collagen - The main supportive protein of skin, tendon, bone, cartilage, and connective tissue.

covalent bond - The linkage between two atoms in which a pair of electrons is shared.

cytochrome P_{450} - A membrane-bound enzyme system involved in the biotransformation of compounds.

cytotoxicity - Specific poisoning action on cells.

direct-acting mutagen - See mutagen.

edema - The accumulation of abnormal amounts of fluid in tissues and organs.

endoplasmic reticulum - A system of channels lined by enzyme-rich membranes within the cell.

epithelium - A continuous layer of cells covering or lining body surfaces, or forming the parenchyma (see definition) of many internal organs.

epoxidation - A reaction in which an oxygen bridge (-O-) is formed between two linked carbon (C) atoms in a hydrocarbon. The resulting compound is called an epoxide.

fibroblast - A connective tissue cell.

frameshift-type mutation - See mutation.

gamete - A reproductive element.

gavage - Feeding through a tube passed into the stomach.

genome - The complete set of hereditary factors as contained in the chromosome (contains the genes).

guanylate cyclase - An enzyme that catalyzes the formation of cyclic GMP (guanosine monophosphate).

hepatocytes - The most predominant type of liver cell.

hilar region - A depressed or pitted part of an organ where the vessels and nerves enter. In the lung, this denotes the region where the two main bronchi enter the main lung lobes.

histopathology - The microscopic analysis of abnormal and diseased tissues.

hyperplasia - The abnormal increase in the number of normal cells in normal arrangements in a tissue.

in vitro tests - Tests carried out within a vessel as opposed to tests in live animals.

in vivo tests - Tests carried out with live animals.

indirect-acting mutagen - See mutagen.

interstitial pneumonitis - An inflammation of lung tissue characterized predominantly by thickening of the alveolar walls.

intratracheal instillation - Application of substances directly into the trachea.

lymph node - A mass of lymphoid tissue that filters lymph (a body fluid) and removes certain foreign agents and bacteria.

lysosomes - Minute bodies found in many types of cells. They contain various hydrolytic enzymes and are normally involved in local cellular digestion.

macrophage - A mobile scavenger cell that is considered to be part of the cell-mediated immune system.

metaplasia - Abnormal cell differentiation characterized by a change of cell type in a tissue to a form not normal for that tissue.

mucociliary escalator - The transport of mucus plus embedded cells and particles from the smaller airways to the pharynx by the ciliated epithelium of the airways (the escalator).

mutagen - A chemical or physical agent that induces genetic mutations.

Direct-acting mutagen - A chemical that without any modification produces mutation.

Indirect-acting mutagen - A chemical that requires metabolic biotransformation into a mutagenic form.

mutation - A permanent transmissible change in the characteristics of an offspring from those of its parents.

Base-pair substitution mutation - A genetic change involving a qualitative substitution of DNA bases.

Frameshift-type mutation - A genetic change involving a quantitative addition or deletion of DNA bases.

necrosis - Cell death.

neoplastic - Pertaining to any new and abnormal growth, such as a tumor.

nitration - The introduction of a nitro ($-NO_2$) group into an organic compound.

nitroarene - See arene.

nitroparaffins - Open-chain, saturated hydrocarbons (alkanes) in which one or more nitro ($-NO_2$) groups has been substituted for a hydrogen atom (H).

nitroreductase - An enzyme that catalyzes the reduction of nitro ($-NO_2$) groups.

nitrosamine - See amines.

o-Methylarylamine - See amines.

ouabain - A glycosidic cardenolide obtained from the seeds of *Strophanthus gratus*. It is used as a cardiotonic.

papilloma - A benign tumor originating in the epithelium (see definition) that is branched or divided into small lobes. It is commonly induced on the skin.

parenchyma - The functional predominant cell type of an organ.

peribronchiolar - Situated around the bronchioles (air passages within the lungs).

peroxide - A compound that contains two oxygen atoms linked together (-O-O-).

phagocytosis - The engulfing of foreign particles by certain cells (phagocytes).

phagolysosomes - A digestive vacuole formed when the membranes of preexistent lysosomes (see definition) within the cytoplasm of a cell merge with the phagosome, a membrane-lined body within a cell containing material taken up by phagocytosis. The lysosomes then discharge the hydrolytic enzymes they contain, which results in the digestion of phagocytized material.

photochemical reaction - A chemical reaction that is stimulated by light radiation. Refers in this report to chemical reactions taking place in the atmosphere that are stimulated by sunlight.

polyaromatic hydrocarbon (PAH) - An organic compound containing more than one 6-membered benzene ring.

primary amine - See amines.

prostaglandin dehydrogenase - An enzyme that catalyzes a transformation of the hormone prostaglandin from one active form to another.

pulmonary emphysema - A chronic lung condition characterized by an increase beyond normal in the size of air spaces and rarefaction of pulmonary tissue, causing shortness of breath.

recombinogenesis - A process or activity that induces exchanges between nonhomologous chromosomes.

revertant - A mutant organism that has undergone a second mutation to reverse the effect of the initial mutagenic event.

S- and *N*-heterocyclic aromatic hydrocarbons - Polyaromatic hydrocarbons (see definition) in which one or more of the rings contains a sulfur (S) or a nitrogen (N) atom replacing a carbon (C) atom.

S9 mix - In the context of this report, a metabolic activation system obtained from the liver by centrifugation and fortified with cofactors.

secondary amines - See amines.

sister chromatid exchange (SCE) - A reciprocal crossing over between chromosomal segments just prior to mitotic separation.

somatic cells - Nonreproductive cells of the body.

spermatocyte - The mother cell of a spermatid (a cell derived from one of the two cells into which a male germ cell divides).

teratogen - An agent or factor that causes malformations (birth defects).
tertiary amine - See amines.
zygote - The fertilized ovum.

REFERENCES

Abraham, W., C. Kim, A. Januszkiewicz, M. Welker, M. Mingle, and R. Schreck (1980). Effects of a Brief Low-Level Exposure to the Particulate Fraction of Diesel Exhaust on Pulmonary Function of Conscious Sheep. Archives of Environmental Health. 35:77-80.

Adamson, I., and D. Bowden (1980). Role of Monocytes and Interstitial Cells in the Generation of Alveolar Macrophages. II. Kinetic Studies after Carbon Loading. Laboratory Investigation. 42:518-524.

American Mining Congress (1978). Health Effects of Diesel Exhaust Emissions: A Comprehensive Literature Review, Evaluation and Research Gaps Analysis. Berkeley, CA: Environmental Health Associates, Inc.

Ames, B. (1979). Identifying Environmental Chemicals Causing Mutagens and Cancer. Science. 204:587-593.

Ames, B., W. Durston, E. Yamasaki, and F. Lee (1973). Carcinogens are Mutagens: A Simple Test System Combining Liver Homogenates for Activation and Bacteria for Detection. Proceedings of the National Academy of Sciences. 70:2281-2285.

Andervont, H., and M. Shimkin (1940). Biologic Testing of Carcinogens. II. Pulmonary Tumor-Induction Technique. Journal of the National Cancer Institute. 1:225-239.

Archer, Z., J. Wagonner, and S. London, Jr. (1973). Uranium Mining and Cigarette Smoking Effects on Man. Journal of Occupational Medicine. 15:204-211.

Asahina, J., A. Carmetl, E. Arnold, Y. Bishop, S. Joshi, D. Coffin, and S. Epstein (1972). Carcinogenicity of Organic Fractions of Particulate Pollutants Collected in New York City and Administered Subcutaneously to Infant Mice. Cancer Research. 32:2263-2268.

Attfield, M. (1978). The Effect of Exposure to Silica and Diesel Exhaust in Underground Metal and Nonmetal Miners. Industrial Hygiene for Mining and Tunneling. Proceedings of the American Conference of Industrial Governmental Hygienists. November 1978. Denver. pp. 129-170.

Barnhart, M., S. Chen, and H. Puro (1979). Impact of Diesel Engine Exhaust (DEE) Particles on the Structural Physiology of the Lung. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Barrett, J., and P. Ts'o (1978). Mechanistic Studies of Neoplastic Transformation of Cells in Culture. Polycyclic Hydrocarbons and Cancer. Volume 2: Molecular and Cell Biology. H. Gelboin and P. Ts'o (eds.). New York: Academic Press. pp. 235-268.

Battigelli, M., R. Mannella, and T. Hatch (1964). Environmental and Clinical Investigation of Workmen Exposed to Diesel Exhaust in Railroad Engine Houses. Industrial Medicine and Surgery. 33:121-124.

Battista, S. (1976). Cilia Toxic Components of Cigarette Smoke. Proceedings of the Third World Conference on Smoking and Health, June 2-5, 1975, New York. E. Wynder, D. Hoffmann, and G. Gori (eds.). 1:517-534.

Beethe, R., R. Wolff, L. Griffis, C. Hobbs, and R. McClellan (1979). Evaluation of a Recently Designed Multi-Tiered Exposure Chamber. Albuquerque: Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute. LF-67, UC-48.

Begeman, D., and R. Klimisch (1977). Tumorigenic Activity of Diesel Exhaust--Results of General Motors/Sloan-Kettering Institute Experiments. Warren, MI: General Motors Research Laboratories, Environmental Science Department. GMR 248C, Rev. 8-77.

Benz, R., and P. Beltz (1979). Testing for the Ability of Marine Diesel Fuel Vapors to Induce Micronuclei on Sister Chromatid Exchanges in Peripheral Lymphocytes Taken from Dogs Exposed Continuously by Inhalation for Thirteen Weeks. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Bingham, E., and H. Falk (1969). Environmental Carcinogens. *Archives of Environmental Health.* 19:779-783.

Bloch, W., T. Lewis, K. Busch, J. Orthoefer, and J. Stara (1972). Cardiovascular Status of Female Beagles Exposed to Air Pollutants. *Archives of Environmental Health.* 24:342-353.

Bouck, N., and G. di Mayorca (1976). Somatic Mutation as the Basis for Malignant Transformation of BHK Cells by Chemical Carcinogens. *Nature.* 264:722-727.

Bradow, R. (March 30, 1980). Gas Phase Mutagens in Diesel Exhaust. U.S. EPA Internal Report to the Office of Mobile Source Air Pollution Control, Washington, D.C.

Brain, J., D. Knudson, S. Sorokin, and M. Davis (1976). Pulmonary Distribution of Particles Given by Intratracheal Instillation or by Aerosol Inhalation. *Environmental Research.* 11:13-33.

Brooks, A., R. Wolff, R. Royer, C. Clark, A. Sanchez, and R. McClellan (1979). Biological Availability of Mutagenic Chemicals Associated with Diesel Exhaust Particles. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Brooks, P. (1977). Role of Covalent Binding in Carcinogenicity. *Biological Reactive Intermediates.* D. Jallow, J. Kocsis, R. Snyder, and H. Vainio (eds.). New York: Plenum Press. pp. 470-480.

Brune, H., M. Habs, and D. Schmal (1978). The Tumor-Producing Effect of Automobile Exhaust Condensate and Fractions Thereof. Part II. Animal Studies. *Journal of Environmental Pathology and Toxicology.* 1:737-746.

Brusick, D. (1977). The Value and Significance of Carcinogenic, Mutagenic and Teratogenic Tests. *Cutaneous Toxicity.* N. Drill and P. Lazar (eds.). New York: Academic Press. pp. 189-201.

Brusick, D. (1978). The Role of Short-Term Testing in Carcinogen Detection. *Chemosphere.* 7(5):413-417.

Brusick, D. (1980). The Consequences of Genotoxic Effects in Humans and Other Mammals. *Principles of Genetic Toxicology.* New York: Plenum Press. pp. 45-68.

Buck, S., and D. Brown (1964). Mortality from Lung Cancer and Bronchitis in Relation to Smoke and SO_2 Concentration, Population Density, and Social Index. Paper prepared for the Tobacco Research

Council, London, England. Research Paper No. 7. 29 pages.

Cadle, S., J. Nebel, and R. Williams (1979). Measurements of Unregulated Emissions from General Motors' Light-Duty Vehicles. Warren, MI: General Motors Research Laboratories, Environmental Science Department. GMR-3019 ENV #63.

Calabrese, E. (1978). Animal Model of Human Disease. Increased Sensitivity to Ozone. Animal Model: Mice with Low Levels of G-6-PD. American Journal of Pathology. 91:409-411.

Campbell, J. (1936). The Effects of Exhaust Gases from Internal Combustion Engines and of Tobacco Smoke Upon Mice, With Special Reference to Tumors of the Lung. British Journal of Experimental Pathology. 17:146-158.

Campbell, K., E. George, and I. Washington, Jr. (1979). Enhanced Susceptibility to Infection in Mice After Exposure to Dilute Exhaust From Light-Duty Diesel Engines. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Cantrell, E., H. Tyrer, W. Perirano, and R. Danner (1979). Benzo[a]pyrene Metabolism in Mice Exposed to Diesel Exhaust. II. Metabolism and Excretion. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Carrano, A., L. Thompson, P. Lindl, and J. Minkler (1978). Sister Chromatid Exchange as an Indicator of Mutagenesis. Nature. 271:551-553.

Casto, B., G. Hatch, S. Huang, J. Huisingh, S. Nesnow, and M. Waters (1979). Mutagenic and Carcinogenic Potency of Extracts of Diesel and Related Environmental Emissions. In Vitro Mutagenesis and Oncogenic Transformation. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Chan, T., P. Lee, and J. Siak (1979). Diesel Particulate Collection for Biological Testing. Comparison of Electrostatic Precipitation and Filtration. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Charboneau, J., and R. McCauley (1979). The Effects of In Vivo Exposure of Rats to Diluted Diesel Exhaust on Microsomal Oxidation of Benzo[a]pyrene. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Chaudhari, A., R. Farmer, and S. Dutta (1979). Effect of Exposure to Diesel Exhaust on Pulmonary Prostaglandin Dehydrogenase (PGDH) Activity. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Chen, S., M. Weller, and M. Barnhart (1980). Effects of Diesel Engine Exhaust on Pulmonary Alveolar Macrophages. Wayne State University School of Medicine. Warren, MI: General Motors Research Laboratories. CR-80-6/B1.

Choudhury, D., and G. Bush (1979). Polynuclear Aromatic Hydrocarbons in Diesel Emission Particulates. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Choudhury, D., and C. Doudney (1979). Isolation of Mutagenic Fractions of Diesel Exhaust Particulates as an Approach to Identification of the Major Constituents. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Claxton, L. (1979). Mutagenic and Carcinogenic Potency of Diesel and Related Environmental Emissions: Salmonella Bioassay. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Claxton, L., and H. Barnes (1980). The Mutagenicity of Diesel Exhaust Exposed to Smog Chamber Conditions as Shown by Salmonella Typhimurium. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Cleaver, J., and D. Bootsma (1975). Xeroderma Pigmentosum: Biochemical and Genetic Characteristics. Annual Reviews of Genetics. H. L. Roman (ed.). Palo Alto, CA: Annual Reviews, Inc. pp. 19-38.

Coffin, D., and E. Blommer (1967). Acute Toxicity of Irradiated Auto Exhaust. Archives of Environmental Health. 15:36-38.

Comité des Constructeurs d'Automobiles du Marché Commun (1980). Inhalation Studies with Gasoline and Diesel Engine Exhaust on Rats and Hamsters. Report to the Health Effects Panel of site visit to the Battelle Institute, Geneva, Switzerland.

Connecticut State Department of Health (1968). Cancer in Connecticut. Survival Experience: 1935-1962. Hartford, CT: State Department of Health.

Curren, R., R. Kouri, C. Kim, and L. Schechtman (1979). Mutagenic and Carcinogenic Potency of Extracts from Diesel and Related Environmental Emissions: Simultaneous Morphological Transformation and Mutagenesis in BALB/c3T3 Cells. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Decoufle, P., K. Stanislawczyk, L. Houten, D. Irwin, J. Bross, and E. Viadana (1977). A Retrospective Survey of Cancer in Relation to Occupation. Cincinnati, OH: National Institute for Occupational Safety and Health. DHEW(NIOSH) Publication No. 77-178.

Deichmann, W., W. MacDonald, W. Lampe, I. Dressler, and W. Anderson (1965). Nitro Olefins as Potential Carcinogens in Air Pollution. Industrial Medicine and Surgery. 34:800-807.

deLamare, P., and J. Ridd (1969). Aromatic Substitution in Bi- and the Polycyclic Systems. Aromatic Substitution, Nitration and Halogenation. New York: Academic Press. pp. 1969-1987.

Department of Health and Social Security of England (1979). A Consultative Document on Guidelines for the Testing of Chemicals for Mutagenicity. Committee on Mutagenicity of Chemicals in Food, Consumer Products, and the Environment. London, England: Department of Health and Social Security.

Doudney, D., M. Franks, and C. Rinaldi (1979). The DNA Damage Activity (DDA) Assay and Its Application to River Waters and Diesel Exhausts. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Dungworth, D., L. Schwartz, W. Tyler, and R. Phalen (1976). Morphological Methods for Evaluation of Pulmonary Toxicity in Animals. Annual Review of Pharmacology and Toxicology. 16:381-399.

El Batawi, M., and M. Noweir (1966). Health Problems Resulting from Prolonged Exposure to Air Pollution in Diesel Bus Garages. *Industrial Health*. 4:1-10.

Evans, M., N. Dekker, L. Cabral-Anderson, and G. Freeman (1978). Quantitation of Damage to the Alveolar Epithelium by Means of Type 2 Cell Proliferation. *American Review of Respiratory Disease*. 118:787-790.

Fahmy, O., and M. Fahmy (1972). Mutagenic Selectivity for the RNA Forming Genes in Relation to the Carcinogenicity of Alkylating Agents and Polycyclic Aromatics. *Cancer Research*. 32:550-557.

Falk, H., P. Kotin, and I. Markul (1958). The Disappearance of Carcinogens From Soot in Human Lungs. *Cancer*. 11:482-489.

Fern, V. (1979). Effects of Exposure to Acetaldehyde in Syrian Hamsters Simultaneously Treated with Benzo[*a*]pyrene or Diethylnitrosamine. *Progress in Experimental Tumor Research*. 24:162-176.

Feron, F., D. De Jong, and P. Emmelot (1973). Dose-Response Correlation for the Induction of Respiratory Tract Tumors in Syrian Golden Hamsters by Intratracheal Instillations of Benzo[*a*]pyrene. *European Journal of Cancer*. 9:387-390.

Ferris, B. (1978). Epidemiology Standardization Project. *American Review of Respiratory Diseases*. 118 (Supplement): 1-20.

Fine, D. (1978). An Assessment of Human Exposure to N-Nitroso Compounds. *Environmental Aspects of N-Nitroso Compounds*. E. Walker, M. Castegnaro, L. Griciute, and R. Lyle (eds.). Lyons, France: International Agency for Research on Cancer. Scientific Publications No. 19. pp. 267-278.

Gertz, E. (1973). Animal Model of Human Disease. Myocardial Failure, Muscular Dystrophy. Animal Model: Cardiomyopathic Syrian Hamster. *American Journal of Pathology*. 70:151-154.

Goldsmith, J., and L. Friberg (1977). Effects of Air Pollution on Human Health. *Air Pollution*. Vol. II. A. Stern (ed.). New York: Academic Press. pp. 457-610.

Goodman, D., P. Hakkinen, J. Nemenzo, and M. Bore (1977). Mutagenic Evaluation of Nitrofuran Derivatives in *Salmonella Typhimurium*, by the Micronucleus Test, and by *In Vivo* Cytogenetics. *Mutation Research*. 48:292-305.

Graf, J. (1979). Preparation and Characterization of Diesel Exhaust Particles for Biological Experiments.

Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Grimmer, G., and H. Bohnke (1978). The Tumor-Producing Effect of Automobile Exhaust Condensate and Fractions Thereof. Part I. Chemical Studies. Journal of Environmental Pathology and Toxicology. 1:661-667.

Gross, K. (1979). Pulmonary Function Testing of Animals Chronically Exposed to Diluted Diesel Exhaust. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Grover, P. (1976). Reactions of Polycyclic Hydrocarbon Metabolites with DNA. In Vitro Metabolic Activation in Mutagenesis Testing. F. deSerres, J. Fouts, J. Bend, and R. Philpot (eds.). Amsterdam: Elsevier/North Holland Biomedical Press. pp. 295-312.

Guerrero, R., D. Rounds, and J. Orthoefer (1979). Sister Chromatid Exchange Analysis of Syrian Hamster Lung Cells Treated In Vivo with Diesel Exhaust Particulates. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Haenszel, W., D. Loveland, and M. Sirken (1962). Lung Cancer Mortality as Related to Residence and Smoking Histories. I. White Males. Journal of the National Cancer Institute. 28: 947-1001.

Haenszel, W., and K. Taeuber (1964). Lung Cancer Mortality as Related to Residence and Smoking Histories. II. White Females. Journal of the National Cancer Institute. 32: 803-838.

Hahn, F., and C. Hobbs (1979). The Effect of Enzyme-Induced Pulmonary Emphysema in Syrian Hamsters on the Deposition and Long-Term Retention of Inhaled Particles. Archives of Environmental Health. 34:203-210.

Hannunkari, I., E. Jarvinen, and T. Partanen (1978). Work Conditions and Health of Locomotive Engineers. II. Questionnaire Study, Mortality and Disability. Scandinavian Journal of Work Environment and Health. 4(Supplement 3):15-28.

Hanson, R., R. Royer, and A. Brooks (1979). Identification of Organic Compounds in Diesel Exhaust Particles. Annual Report of the Inhalation Toxicology Research Institute Operated for the U.S.

Department of Energy By the Lovelace Biomedical and Environmental Research Institute, Inc. R. Henderson, J. Diel, and B. Martinez (eds.). Albuquerque. pp. 203-206. LF-69, UC-48.

Hare, C., and T. Baines (1979). Characterization of Particulate and Gaseous Emissions from Two Diesel Automobiles as Functions of Fuel and Driving Cycle. SAE Technical Paper Series. Publication No. 790424.

Harris, J. (1981). Estimate of the Potential Risk of Lung Cancer from Diesel Engine Emissions. Report prepared for the Diesel Impacts Study Committee, National Research Council. Forthcoming.

Haschek, W., and H. Witschi (1979). Pulmonary Fibrosis--A Possible Mechanism. Toxicology and Applied Pharmacology. 51:475-487.

Hayashi, C. (1961). Theory of Quantification and its Examples. V. Proceedings of the Institute of Statistical Mathematics. 8:149-151.

Heinrich, U., W. Stober, and F. Pott (1979). Long Term Diesel Exhaust Inhalation Studies with Hamsters. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Henry, J., C. Port, and D. Kaufman (1974). Role of Particles in Respiratory Carcinogenesis Bioassays. Experimental Lung Cancer, Carcinogenesis and Bioassays. E. Karbe and F. Park (eds.). New York: Springer-Verlag. pp. 161-172.

Heppleston, A. (1975). Animal Model of Human Disease. Pulmonary Alveolar Lipo-Proteinosis. Animal Model: Silica-Induced Pulmonary Alveolar Lipo-Proteinosis. American Journal of Pathology. 78:171-174.

Hoffmann, D., E. Theisz, and E. Wynder (1965). Studies on the Carcinogenicity of Gasoline Exhaust. Journal of the Air Pollution Control Association. 15:162-165.

Hoffmann, D., A. Rivenson, S. Hecht, J. Hilfrich, H. Kobayashi, and E. Wynder (1979). Model Studies in Tobacco Carcinogenesis with the Syrian Golden Hamster. Progress in Experimental Tumor Research. 24:370-390.

Hollstein, M., J. McCann, F. Angelosanto, and W. Nichols (1979). Short-Term Tests For Carcinogens and Mutagens. Mutation Research. 65:133-226.

Holt, P., D. Keast, and J. MacKenzie (1978). Animal Model of Human Disease. Infectious and Neoplastic Respiratory Diseases Associated with Cigarette

Smoking. Animal Model: Immunosuppression in the Mouse Induced by Long-Term Exposure to Cigarette Smoke. American Journal of Pathology. 90:281-284.

Hueper, W. (1955). A Quest Into the Environmental Causes of Cancer of the Lung. Washington, D.C.: U.S. Department of Health, Education, and Welfare, Public Health Service. Public Health Monograph No. 36.

Huisingh, J., R. Bradow, R. Jungers, L. Claxton, R. Zweidinger, S. Tejada, J. Bumgarner, F. Duffield, M. Waters, V. Simmon, C. Hare, C. Rodriguez, and L. Snow (1978). Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures. M. Waters, S. Nesnow, J. Huisingh, S. Sandhu, and L. Claxton (eds.). Research Triangle Park, NC: EPA, Health Effects Research Laboratory. EPA-600/9-78-027.

Huisingh, J., R. Bradow, R. Jungers, B. Harris, R. Zweidinger, K. Cushing, B. Gill, and R. Albert (1979). Mutagenic and Carcinogenic Potency of Extracts of Diesel and Related Environmental Emissions: Study Design, Sample Generation, Collection, and Preparation. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Hyde, D., J. Orthoefer, D. Dungworth, W. Tyler, R. Carter, and H. Lum (1978). Morphometric and Morphologic Evaluation of Pulmonary Lesions in Beagle Dogs Chronically Exposed to High Ambient Levels of Air Pollutants. Laboratory Investigation. 38:455-469.

Interagency Regulatory Liaison Group (1979). Scientific Bases for Identifying Potential Carcinogens and Estimating Their Risks. Report of the Work Group on Risk Assessment. February 6, 1979.

International Agency for Research on Cancer (1976a). Screening Tests in Chemical Carcinogenesis. Proceedings of a Workshop held in Brussels, Belgium. June 1975. Lyon: International Agency for Research on Cancer. IARC Scientific Publication No. 12.

International Agency for Research on Cancer (1976b). IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man: Cadmium, Nickel, Some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatile Anaesthetics. Volume 11. Lyon: International Agency for Research on Cancer.

International Agency for Research on Cancer (1977). Air Pollution and Cancer in Man. Proceedings of the Second Hanover International Carcinogenesis Meeting. October 1975. Lyon: International Agency for Research on Cancer. IARC Scientific Publication No. 16.

International Commission on Radiological Protection (1966). Deposition and Retention Models for Internal Dosimetry of the Human Respiratory Tract. Report prepared by the Task Group on Lung Dynamics. Health Physics. 12:173-207.

International Union Against Cancer (1976). Animal Models. Lung Cancer. A Series of Workshops on the Biology of Human Cancer. Geneva: UICC Technical Report Series. Report #3. 25:1-130.

Jorgensen, H., and A. Svensson (1970). Studies on Pulmonary Function and Respiratory Tract Symptoms of Workers in an Iron Ore Mine Where Diesel Trucks are Used Underground. Journal of Occupational Medicine. 12:349-354.

Kalter, H. (1971). Correlation Between Teratogenic and Mutagenic Effects of Chemicals in Mammals. Chemical Mutagens: Principles and Methods for Their Detection. Volume I. A. Hollaender (ed.). New York: Plenum Press. pp. 57-82.

Kaplan, I. (1959). Relationship of Noxious Gases to Carcinoma of the Lung in Railroad Workers. Journal of the American Medical Association. 171:2039-2043.

Karlinsky, J., and G. Snider (1978). Animal Models of Emphysema. American Review of Respiratory Disease. 117:1109-1133.

Kentera, D., D. Susic, M. Zdravkovic, V. Kanjuh, and G. Tucakovic (1978). Chronic Pulmonary Hypertension. Comparative Pathology Bulletin. 10:2,4.

King, L., M. Kohan, A. Austin, L. Claxton, and J. Huisingh (1980). Evaluation of the Release of Mutagens from Diesel Particulates in the Presence of Physiological Fluids. Draft of Manuscript in preparation. Research Triangle Park, NC: EPA, Health Effects Research Laboratory.

Kotin, P., H. Falk, P. Mader, and M. Thomas (1954a). Aromatic Hydrocarbons. I. Preserve in a Los Angeles Atmosphere and the Carcinogenicity of Atmosphere Extracts. AMA Archives of Industrial Hygiene and Occupational Medicine. 9:153-163.

Kotin, P., H. Falk, and M. Thomas (1954b). Aromatic Hydrocarbons. II. Presence in the Particulate

Phase of Gasoline-Engine Exhausts and the Carcinogenicity of Exhaust Extracts. *AMA Archives of Industrial Health*. 9:164-177.

Kotin, P., H. Falk, and M. Thomas (1955). Aromatic Hydrocarbons. III. Presence in the Particulate Phase of Diesel-Engine Exhausts and the Carcinogenicity of Exhaust Extracts. *AMA Archives of Industrial Health*. 11:113-120.

Kotin, P., and H. Falk (1963). Atmospheric Factors in Pathogenesis of Lung Cancer. *Advances in Cancer Research*. 7:475-514.

Lane, D., and M. Katz (1977). The Photomodification of Benzo[*a*]pyrene, Benzo[*b*]fluoranthene, and Benzo[*k*]fluoranthene Under Simulated Atmospheric Conditions. *Fate of Pollutants in the Air and Water Environments*. Part 2. I. Suffet (ed.). New York: John Wiley and Sons, Inc. pp. 137-154.

Laskin, S., and A. Sellakumar (1974). Models in Chemical Respiratory Carcinogenesis. *Experimental Lung Cancer, Carcinogenesis and Bioassays*. E. Karbe and F. Park (eds.). New York: Springer-Verlag. pp. 7-19.

Laurie, R., and W. Boyes (1979). Neurophysiological Alterations Due to Diesel Exhaust Exposure During the Neonatal Life of the Rat. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Laurie, R., W. Boyes, and T. Wessendarp (1979). Behavioral Alterations Due to Diesel Exhaust Exposure. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Lawley, P. (1976). Carcinogenesis by Alkylating Agents. *Chemical Carcinogens*. C. E. Searle (ed.). Washington, D.C.: American Chemical Society Monograph. 173:83-244.

Lee, I., K. Suzuki, S. Lee, and R. Dixon (1980). Aryl Hydrocarbon Hydroxylase Induction in Rat Lung, Liver and Male Reproductive Organs Following Inhalation Exposure to Diesel Emission. *Toxicology and Applied Pharmacology*. 52:181-184.

Lee, S., M. Malanchuk, and V. Finellie (1976). Biologic Effects of Auto Emissions. I. Exhaust From Engine With and Without Catalytic Converter. *Journal of Toxicology and Environmental Health*. 1:705-712.

Leupker, R., and M. Smith (1978). Mortality in Unionized Truck Drivers. Journal of Occupational Medicine. 20:677-682.

Liber, J., B. Andon, R. Hites, and W. Thilly (1979). Diesel Soot: Mutation Measurements in Bacterial and Human Cells. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Lofroth, G. (1979). Salmonella/Microsome Mutagenicity Assays of Exhaust from Diesel and Gasoline Powered Motor Vehicles. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Loprieno, N., F. DeLorenzo, G. Cornetti, and G. Biaggini (1979). Mutagenicity Studies on Diesel Particles and Particulate Extracts. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Lutz, W. (1979). In Vivo Covalent Binding of Organic Chemicals to DNA as a Quantitative Indicator in the Process of Carcinogenesis. Mutation Research. 65:289-356.

Lyons, M. (1962). Comparison of Aromatic Polycyclic Hydrocarbons from Gasoline Engine and Diesel Engine Exhaust, General Atmospheric Dust, and Cigarette Smoke Condensate. Paper presented at the Symposium on the Analysis of Carcinogenic Air Pollutants. August 1961. Cincinnati. National Cancer Institute Monograph Number 9. pp. 193-199.

McCann, J., E. Choi, E. Yamasaki, and B. Ames (1975). Detection of Carcinogens as Mutagens in the Salmonella/Microsome Test: Assay of 300 Chemicals. Proceedings of the National Academy of Sciences. 72:5135-5139.

McCormick, J., R. Zator, B. DaGue, and V. Maher (1979a). Diesel Exhaust Particulate Causes Enhanced Cell Killing in DNA Repair Deficient Human Fibroblasts. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

McCormick, J., R. Zator, B. DaGue, and V. Maher (1979b). Studies on the Effects of Diesel Particulate on Normal and Xeroderma Pigmentosum Cells. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Magee, P. (1977). The Relationship Between Mutagenesis, Carcinogenesis and Teratogenesis. Progress in Genetic Toxicology. D. Scott, B. Bridges, and F. Sobels (eds.). Amsterdam: Elsevier/North Holland Biomedical Press. pp. 15-27.

Magee, P., R. Montesano, and R. Preussmann (1976). N-Nitroso Compounds and Related Carcinogens. Chemical Carcinogens. C. Searle (ed.). Washington, D.C.: American Chemical Society Monograph. 173:491-625.

Menck, H., and B. Henderson (1976). Occupational Differences in Rates of Lung Cancer. Journal of Occupational Medicine. 18:797-801.

Mentser, M., and A. Sharkey (1977). Chemical Characterization of Diesel Exhaust Particulates. Pittsburgh, PA: U.S. Energy Research and Development Administration. PERC/RI-77/5.

Milby, T., D. Whorton, and D. Lassiter (1979). Diesel Exhaust Exposure--Human Health Effects Research Recommendations. Report of Environmental Health Associates, Inc., to the Coordinating Research Council, Inc.

Miller, E., and J. Miller (1971). The Mutagenicity of Chemical Carcinogens: Correlations, Problems and Interpretations. Chemical Mutagens: Principles and Methods for Their Detection. Volume 1. A. Hollaender (ed.). New York: Plenum Press. pp. 83-119.

Misfeld, J. (1979). The Tumor Producing Effects of Automobile Exhaust Condensate and of Diesel Exhaust Condensate. Mathematical-Statistical Evaluation of the Test Results. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Misfeld, J., and J. Timm (1978). The Tumor-Producing Effects of Automobile Exhaust Condensate and Fractions Thereof. Part III. Mathematical-Statistical Evaluation of the Test Results. Journal of Environmental Pathology and Toxicology. 1:747-772.

Mishra, N., and G. di Mayorca (1974). In Vitro Malignant Transformation of Cells by Chemical Carcinogens. Biochemical Biophysical Acta. 355:205-219.

Misiorowski, R., K. Strom, J. Vostal, and M. Chvapil (1979). Lung Biochemistry of Rats Chronically

Exposed to Diesel Particulates. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Mitchell, A., E. Evans, M. Jotz, E. Riccio, K. Mortelmans, and V. Simmon (1979). Mutagenic and Carcinogenic Potency of Extracts of Diesel and Related Environmental Emissions: *In Vitro* Mutagenesis and DNA Damage. Paper presented at the EPA International Symposium on the Health Effects of Diesel Emissions. December 1979. Cincinnati.

Mittler, S., and S. Nicholson (1957). Carcinogenicity of Atmospheric Pollutants. *Industrial Medicine and Surgery*. 26:135-138.

Moore, W., J. Orthoefer, J. Burkart, and M. Malanchuk (1978). Preliminary Findings of the Deposition and Retention of Automotive Diesel Particulate in Rat Lungs. Paper presented at the 71st Annual Meeting of the Air Pollution Control Association. June 1978. Houston.

Mustafa, G., and D. Tierney (1978). Biochemical and Metabolic Changes in the Lung With Oxygen, Ozone, and Nitrogen Dioxide Toxicity. *American Review of Respiratory Disease*. 118:1061-1090.

National Cancer Institute (1976). *Guidelines for Carcinogen Bioassay in Small Rodents*. DHEW (NIH) Publication No. 76-801.

National Research Council (1972). *Particulate Polycyclic Organic Matter. Report of the Committee on Biologic Effects of Atmospheric Pollutants*. Washington, DC: National Academy of Sciences.

National Research Council (1975). *Nickel. Report of the Committee on Medical and Biologic Effects of Environmental Pollutants*. Washington, DC: National Academy of Sciences.

National Research Council (1976). *Vapor Phase Organic Pollutants. Report of the Committee on Medical and Biologic Effects of Environmental Pollutants*. Washington, DC: National Academy of Sciences.

National Research Council (1977a). *Nitrogen Oxides. Report of the Committee on Medical and Biologic Effects of Environmental Pollutants*. Washington, DC: National Academy of Sciences.

National Research Council (1977b). *Ozone and Other Photochemical Oxidants. Report of the Committee on Medical and Biologic Effects of Environmental*

Pollutants. Washington, DC: National Academy of Sciences.

National Research Council (1978). Sulfur Oxides. Report of the Committee on Sulfur Oxides. Board on Toxicology and Environmental Health Hazards. Washington, DC: National Academy of Sciences.

Nesnow, S., and J. Huisingh (1979). Mutagenic and Carcinogenic Potency of Extracts of Diesel and Related Environmental Emissions: Summary and Discussion of the Results. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Nettesheim, P., and R. Griesemer (1978). Experimental Models for Studies of Respiratory Tract Carcinogenesis. Pathogenesis and Therapy of Lung Cancer. Lung Biology in Health and Disease. Volume 10. C. Harris (ed.). New York: Marcel Dekker, Inc. pp. 75-188.

Ohnishi, Y., K. Kosei, K. Sato, I. Tahara, H. Takeyyoshi, and H. Tokiva (1980). Detection of Mutagenic Activity in Automobile Exhaust. Mutation Research. 77:229-240.

Orthoefer, J., R. Bhatnagar, A. Rahman, Y. Yang, S. Lee, and J. Stara (1976). Collagen and Prolyl Hydroxylase Levels in Lungs of Beagles Exposed to Air Pollutants. Environmental Research. 12:299-305.

Orthoefer, J., W. Moore, D. Kraemer, F. Truman, W. Crocker, and Y. Yang (1979). Carcinogenicity of Diesel Exhaust as Tested In Strain 'A' Mice. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Pattle, R., H. Stretch, F. Burgess, K. Sinclair, and J. Edginton (1957). The Toxicity of Fumes From a Diesel Engine Under Four Different Running Conditions. British Journal of Industrial Medicine. 14:47-55.

Pederson, T. (1979). DNA-Binding Studies With Diesel Exhaust Particle Extract. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Pederson, T. (1980). Characterization of Direct Acting Mutagens in Diesel Particulates by Thin Layer Chromatography. Warren, MI: General Motors Research Laboratories, Biomedical Science Department. GMR-3265.

Pepelko, W., J. Mattox, W. Moorman, and J. Clark (1979a). Pulmonary Function Evaluation of Cats After One Year of Exposure to Diesel Exhaust. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Pepelko, W., A. Vinegar, and A. Carson (1979b). Pulmonary Function Changes in Chinese Hamsters Exposed Six Months to Diesel Exhaust. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Pereira, M., P. Sabharwal, P. Kaur, C. Ross, A. Choi, and T. Dixon (1979a). In Vivo Detection of Mutagenic Effects of Diesel Exhaust by Short-Term Mammalian Bioassays. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Pereira, M., P. Sabharwal, L. Gordon, and A. Wyrobek (1979b). The Effect of Diesel Exhaust on Sperm-Shape Abnormalities in Mice. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Pereira, M., T. Connor, J. Meyne, and M. Legator (1979c). Metaphase Analysis, Micronuclei Assay and Urinary Mutagenicity Assay of Mice Exposed to Diesel Emissions. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Pereira, M., H. Shinozuka, and B. Lombardi (1979d). Test of Diesel Exhaust Emissions in the Rat Liver Foci Assay. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Pitts, J. (1979). Photochemical and Biological Implications of Atmospheric Reactions of the Amines and Benzo[a]pyrenes. Philosophical Transactions of the Royal Society of London. A290:551-576.

Pitts, J., D. Grosjean, K. Van Cauwenberghe, J. Schmid, and D. Fitz (1978a). Photooxidation of Aliphatic Amines Under Simulated Atmospheric Conditions: Formation of Nitrosamines, Nitramines, Amides, and Photochemical Oxidants. Environmental Science and Technology. 12:946-953.

Pitts, J., K. Van Cauwenberghe, D. Grosjean, J. Schmid, D. Fitz, W. Belser, Jr., G. Knudson, and P. Hynds

(1978b). Atmospheric Reactions of Polycyclic Aromatic Hydrocarbons: Acyl Formation of Mutagenic Nitro Derivatives. Science. 202:515-519.

Pitts, J., A. Winer, D. Lokensgard, S. Shaffer, E. Tuazon, and G. Harris (1979). Interactions Between Diesel Emissions and Gaseous Co-Pollutants in Photochemical Air Pollution: Some Health Implications. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Puro, H. (1980). Light Microscopic Findings in Lungs of Rats and Guinea Pigs Exposed to Diesel Exhaust. Wayne State University School of Medicine. Wayne, MI: General Motors Research Laboratories. CR-80-7/B1.

Raffle, P. (1957). The Health of the Worker. British Journal of Industrial Medicine. 14:73-80.

Reger, R., and J. Hancock (undated). On Respiratory Health. Coal Miners Exposed to Diesel Exhaust Emissions. Morgantown, WV: Appalachian Laboratory for Occupational Safety and Health.

Reger, R., J. Hankinson, and J. Merchant (undated). Ventilatory Function Changes Over a Work Shift for Coal Miners Exposed to Diesel Emissions. Morgantown, WV: Appalachian Laboratory for Occupational Safety and Health.

Risby, T., R. Yaskin, and S. Lestz (1979). Diesel Particulate Matter. Chemical and Biological Assays. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Risby, T., and J. Sigsby (1980). Exhaust Emissions From a Diesel Engine. Annual Report on U.S. Environmental Protection Agency Grant No. R-806558.

Rockette, H. (1977). Mortality Among Coal Miners Covered by the UMWA Health and Retirement Funds. U.S. Department of Health, Education, and Welfare, National Institute for Occupational Safety and Health. DHEW (NIOSH) Publication No. 77-155.

Rodriguez, T., J. Fisher, and D. Johnson (1979). Characterization of Organic Constituents in Diesel Exhaust Particulates. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Royal College of Physicians (1970). Air Pollution and Lung Cancer. Air Pollution and Health. London:

Pitman Medical and Scientific Publishing Company, Ltd. pp. 48-57.

Rudd, C. (1979). Diesel Particulate Extracts in Cultured Mammalian Cells. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Saffiotti, U., R. Montesano, A. Sellakumar, and D. Kaufman (1972). Respiratory Tract Carcinogenesis Induced In Hamsters by Different Dose Levels of Benzo[a]pyrene and Ferric Oxide. Journal of the National Cancer Institute. 49:1199-1204.

Santodonato, J., D. Basu, and P. Howard (1978). Health Effects Associated with Diesel Exhaust Emissions. Literature Review and Evaluation. Springfield, VA: National Technical Information Service. EPA-600/1-78-063.

Sawicki, E., W. Elbert, T. Hauser, F. Fox, and T. Stanley (1960). Benzo[a]pyrene Content of the Air of American Communities. American Industrial Hygiene Association Journal. 21:443-451.

Schenker, M., and J. Speizer (1979). A Retrospective Cohort Study of Diesel Exhaust Exposure in Railroad Workers: Study Design and Methodologic Issues. Paper presented at the EPA International Symposium on the Health Effects of Diesel Emissions. December 1979. Cincinnati.

Schneider, D., and B. Felt (1979). Effect of Diesel Particulate Exposure on Adenylate and Guanylate Cyclase of Rat and Guinea Pig Liver and Lung. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Schreck, R., S. Soderholm, T. Chan, W. Hering, J. D'Arcy, and K. Smiler (1979). Introduction to and Experimental Conditions in the General Motors Research Laboratories Chronic Inhalation Studies of Diesel Exhaust. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Schuetzle, D., F. Lee, T. Prater, and S. Teyada (1980). The Identification of Polynuclear Aromatic Hydrocarbon Derivatives in Mutagenic Fractions of Diesel Particulate Extracts. Paper presented at the Tenth Annual Symposium on the Analytical Chemistry of Pollutants, May 1980. Dortmund, Germany. Submitted for publication to *International Journal of Environmental Analytical Chemistry*.

Schuler, R., and R. Niemeier (1979). A Study of Diesel Emissions on Drosophila. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Selikoff, I., E. Hammond, and J. Chung (1968). Asbestos Exposure, Smoking and Neoplasia. *Journal of the American Medical Association*. 204:106-112.

Selikoff, I., H. Seidman, and E. Hammond (1980). Mortality Effects of Cigarette Smoking Among Amocite Asbestos Workers. *Journal of the National Cancer Institute*. 65:507-513.

Setlow, R. (1978). Repair Deficient Human Disorders and Cancer. *Nature*. 271:713-717.

Shefner, A., L. Dooley, A. Files, C. Grubbs, and J. Rust (1979). Carcinogenicity of Diesel Exhaust Particles by Intratracheal Instillation--Dose Range Study. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Shimkin, M., and G. Stoner (1975). Lung Tumors in Mice: Application to Carcinogenesis Bioassay. *Advances in Cancer Research.* G. Klein and S. Weinhouse (eds.). New York: Academic Press. 21:1-58.

Siak, J., T. Chan, and P. Lee (1979). Diesel Particulate Extracts in Bacterial Test Systems. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Slaga, T., L. Triplett, and S. Nesnow (1979). Mutagenic and Carcinogenic Potency of Extracts of Diesel and Related Environmental Emissions: Two-Stage Carcinogenesis in Skin Tumor Sensitive Mice (Sencar). Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Snider, G., J. Hayes, and A. Korthy (1978). Chronic Interstitial Pulmonary Fibrosis Produced in Hamsters by Endotracheal Bleomycin. *American Review of Respiratory Disease*. 117:1099-1108.

Soderholm, S. (1979). Physical Characterization of Diesel Exhaust Particles in Exposure Chambers. Paper presented at the EPA International Symposium on the Health Effects of Diesel Emissions. December 1979. Cincinnati.

Stara, J., W. Moore, and A. Breidenbach (1974). Toxicology of Atmospheric Pollutants Resulting From Fuel Additives and Emissions Associated with the Use of Automobile Catalytic Converters. *International Symposium Proceedings on Recent Advances in the Assessment of the Health Effects of Environmental Pollution*. June 1974. Paris. Luxembourg: Commission of the European Communities pp. 751-772.

Stoeber, W., U. Mohr, and J. Althoff (1980). Inhalation Studies with Syrian Golden Hamsters, Mice and Rats. Report to the Health Effects Panel on site visit to the Fraunhofer Institute, University of Muenster. June 5, 1980.

Stupfel, M. (1976). Recent Advances in Investigations of Toxicity of Automotive Exhaust. *Environmental Health Perspectives*. 17:253-285.

Stupfel, M., M. Magnier, F. Romary, M. Tran, and J. Moutet (1973). Lifelong Exposure of SPF Rats to Automotive Exhaust Gas. *Archives of Environmental Health*. 26:264-269.

Swenberg, J., W. Kerns, R. Mitchell, E. Gralla, and K. Pavkov (1980). Induction of Squamous Cell Carcinomas of the Rat Nasal Cavity by Inhalation Exposure to Formaldehyde Vapor. *Cancer Research*. 40:3398-3402.

Tomatis, L., C. Partensky, and R. Montesano (1973). The Predictive Value of Mouse Liver Tumor Induction in Carcinogenicity Testing - A Literature Survey. *International Journal of Cancer*. 12:1-20.

U.S. DHEW (1978). Study of the Combined Effects of Smoking and Inhalation of Uranium Ore Dust, Radon Daughters and Diesel Oil Exhaust Fumes in Hamsters and Dogs. Report of the Pacific Northwest Laboratory of the Battelle Memorial Institute to the National Institute of Environmental Health Sciences. Springfield, VA: NTIS. PNL-2744/UC-48.

U.S. EPA (1977). Precautionary Notice on the Handling of Exhaust Products From Diesel Engines. Issued under the signature of S. Gage, Office of Research and Development, Washington, DC. November 4, 1977.

U.S. EPA (1979a). Raw Diesel Exhaust Particulate Exposure of Hamsters for Carcinogenesis. Paper presented at a meeting of the Health Effects Panel, Diesel Impacts Study Committee, National Academy of

Sciences, June 30-July 1, 1979, Woods Hole, Massachusetts, by W. Pepelko, Health Effects Research Laboratory, Office of Research and Development, Cincinnati, OH.

U.S. EPA (1979b). Test of Carcinogenicity by Lung Tumor Response in Strain 'A' Mice. Paper submitted at a meeting of the Health Effects Panel, Diesel Impacts Study Committee, National Academy of Sciences, June 30-July 1, 1979, Woods Hole, Massachusetts, by W. Pepelko, Health Effects Research Laboratory, Office of Research and Development, Cincinnati, OH.

U.S. EPA (1979c). Assay of Carcinogenic Potency of Drinking Water and Diesel Exhaust: Safe Drinking Water Act and Clean Air Act. Paper presented at a meeting of the Health Effects Panel, Diesel Impacts Study Committee, National Academy of Sciences, June 30-July 1, 1979, Woods Hole, Massachusetts, by W. Pepelko, Health Effects Research Laboratory, Office of Research and Development, Cincinnati, OH.

U.S. EPA (1979d). Confirmatory Mutagenesis Bioassay Data on Extracts from Diesel Particulate. Report of January 29, 1979. Presented at a meeting of the Health Effects Panel, Diesel Impacts Study Committee, National Academy of Sciences, June 30-July 1, 1979, Woods Hole, Massachusetts, by J. Huisingh, Health Effects Research Laboratory, Research Triangle Park, NC.

U.S. EPA (1979e). Study of the Teratologic Effects of Long-Term Exposure to Diesel Exhaust Emissions (Rabbits). Health Effects Research Laboratory, Office of Research and Development. Cincinnati, OH.

U.S. EPA (1979f). Reproduction and Carcinogenesis. Paper presented at a meeting of the Health Effects Panel, Diesel Impacts Study Committee, National Academy of Sciences, June 30-July 1, 1979, Woods Hole, Massachusetts, by W. Pepelko, Health Effects Research Laboratory, Office of Research and Development, Cincinnati, OH.

U.S. EPA (1980). Fuel Economy and Emissions Performance of Diesel Automobiles (FEEPDA). Draft interim report to the Mobile Sources Research Branch by the New York State Department of Environmental Conservation, Division of Air, Bureau of Developmental Technology, Automotive Emissions Evaluation Section. USEPA Grant No. R805934020.

Van Duuren, B., and B. Goldschmidt (1976). Carcinogenic and Tumor-Promoting Agents in Tobacco Carcinogenesis. Journal of the National Cancer Institute. 56:1237-1242.

Vaughan, T., L. Jennelle, and T. Lewis (1969). Long-Term Exposure to Low Levels of Air Pollutants: Effects on Pulmonary Function in the Beagle. Archives of Environmental Health. 19:45-50.

Vos, J. (1977). Immune Suppression as Related to Toxicology. CRC Critical Reviews in Toxicology. 5:67-101.

Vostal, J., T. Chen, B. Garg, P. Lei, and K. Strom (1979). Lymphatic Transport of Inhaled Diesel Particles in the Lungs of Guinea Pigs Exposed to Diluted Diesel Exhaust. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Vuk, D., M. Jones, and J. Johnson (1979). The Measurement and Analysis of the Physical Character of Diesel Particulate Emissions. SAE Technical Paper Series Publication No. 760131.

Waller, R. (1967). Bronchi and Lungs - Air Pollution. The Prevention of Cancer. R. Raven and F. Coe (eds.) London: Butterworth. pp. 181-186.

Waller, R. (1979). Trends in Lung Cancer in London in Relation to Exposure to Diesel Fumes. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Ward, J., R. Greisemer, and E. Weisburger (1979). The Mouse Liver Tumor as an Endpoint in Carcinogenesis Tests. Toxicology and Applied Pharmacology. 51:389-397.

Waxweiler, R., J. Wagoner, and W. Archer (1973). Mortality of Potash Workers. Journal of Occupational Medicine. 15:486-489.

Wei, E., Y. Wang, and S. Rappaport (1980). Diesel Emissions and the Ames Test: A Commentary. Journal of the Air Pollution Control Association. 30:267-271.

Weller, M., S. Chen, and M. Barnhart (1980). Acid Phosphatase in Alveolar Macrophages Exposed In Vivo to Diesel Engine Exhaust. Wayne State University School of Medicine. Warren, MI: General Motors Research Laboratories. CR-80-5/B1.

WHO (1978a). Inhalation Exposure. Environmental Health Criteria: Principles and Methods for Evaluating the Toxicity of Chemicals. Part I. Geneva: World Health Organization. 6:199-234.

WHO (1978b). Acute, Subacute, and Chronic Toxicity Tests. Environmental Health Criteria: Principles and Methods for Evaluating the Toxicity of Chemicals. Part I. Geneva: World Health Organization. 6:95-114.

Williams, G. (1977). Detection of Chemical Carcinogens by Unscheduled DNA Synthesis in Rat Liver Primary Cell Culture. Cancer Research. 37:1845-1851.

Williams, R., and C. Begeman (1979). Characterization of Exhaust Particulate Matter from Diesel Automobiles. Warren, MI: General Motors Research Laboratories, Environmental Science Department. GMR-2970. ENV #61.

Williams, R., and D. Chock (1979). Characterization of Diesel Particulate Exposure. Paper presented at the EPA International Symposium on the Health Effects of Diesel Engine Emissions. December 1979. Cincinnati.

Williams, R., and S. Swarin (1979). Benzo[a]pyrene Emissions from Gasoline and Diesel Automobiles. Warren, MI: General Motors Research Laboratories. Environmental Science and Analytic Chemistry Departments. GMR-2881R.

Wilson, A., R. Fairshter, J. Gillespie, and J. Hackney (1976). Evaluation of Abnormal Lung Function. Annual Review of Pharmacology. 16:465-486.

Witschi, H., and M. Cote (1977). Primary Pulmonary Responses to Toxic Agents. CRC Critical Reviews in Toxicology. 5:23-66.

Wynder, E., and D. Hoffmann (1962). A Study of Air Pollution Carcinogenesis. III. Carcinogenic Activity of Gasoline Engine Exhaust Condensate. Cancer. 15:103-108.

Yamazaki, K. (1969). The Effects of Diesel Exhaust Gas on the Body. Report No. 2. An Analysis of Pulmonary Function Tests. Railway Labor Science. 23:1-11.

Zbinden, G. (1973). Progress in Toxicology, Volume I. New York: Springer-Verlag.

Zweidinger, R., S. Tejada, D. Dropkin, J. Huisingh, and L. Claxton (1980a). Characterization of Extractable Organics in Diesel Exhaust Particulates. Symposium on Diesel Particulate Emissions and

Measurements Characterization. Research Triangle Park, NC.

Zweidinger, R., S. Tejada, D. Dropkin, and F. Black (1980b). Collection and Characterization of Diesel Exhaust Particulate Bound Organics. Draft report. Research Triangle Park, NC: U.S. EPA.

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